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Age, Growth, and Reproductive Biology of Blackcheek Tonguefish, Symphurus plagiusa (Cynoglossidae: Pleuronectiformes), in Chesapeake Bay, Virginia

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A Thesis
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The Faculty of the School of Marine Science
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In Partial Fulfillment
of the Requirements for the Degree of
Master of Arts

By

Mark R. Terwilliger

1996

This thesis is submitted in partical fulfillment of
the requirements for the degree of

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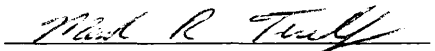
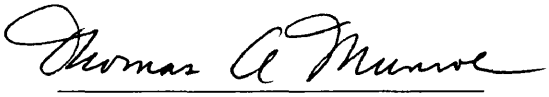


Mark R. Terwilliger

Approved, December 1996



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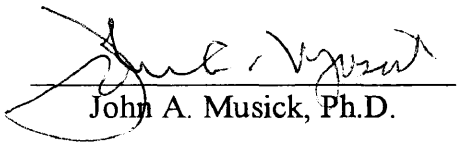
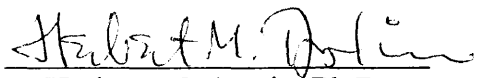


John A. Musick, Ph.D.



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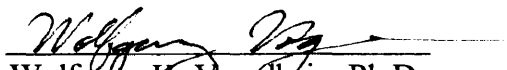

Wolfgang K. Vogelbein, Ph.D.

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ABSTRACT

This study was undertaken to determine age structure, longevity, mortality, spatial and temporal spawning patterns, fecundity, size and age at maturity, and to detail both microscopic and macroscopic gonadal development for a population of blackcheek tonguefish, *Symphurus plagiusa*, in Chesapeake Bay. Blackcheek tonguefish (36-202 mm TL) were randomly collected via otter trawl in lower Chesapeake Bay and the major Virginia tributaries (James, York, and Rappahannock rivers) from April 1994 to August 1995. Ages were determined by thin transverse sections of sagittal otoliths. Marginal increment analysis shows that a single annulus is formed each year in June. Blackcheek tonguefish reached a maximum age of 5+ years. Growth is rapid during the first year, then slows rapidly at a time coincident with maturation. The von Bertalanffy growth equation for males was $L_t = 196.5(1\text{-}e^{-.2853(t+.9195)})$ and for females was $L_t = 190.6(1\text{-}e^{-.3205(t+.7842)})$. Instantaneous mortality rates were 0.87 (Hoenig's equation), 0.92 (Royce's equation), and 1.17 (catch curve). High instantaneous total mortality may reflect either loss due to emigration of adults from Chesapeake Bay onto the continental shelf or natural mortality resulting from environmental stress near the northern range limit.

Blackcheek tonguefish are multiple spawners with asynchronous oocyte development and indeterminate fecundity. Mean length at first maturity for males and females was 91 mm and 101 mm TL, respectively, corresponding to a large age 0 or small age 1 fish. Size-specific sex ratios, date of capture, and location of capture show that mature female blackcheek tonguefish in spawning condition were taken mostly in the deeper, more saline areas of lower Chesapeake Bay. Histological and macroscopic examination of gonads and mean GSI values indicate that blackcheek tonguefish have an extended reproductive season in Chesapeake Bay, extending from June through early October. Batch fecundity of blackcheek tonguefish ranging from 116 mm to 175 mm TL ranged from 2846 to 16881 ova. Batch fecundity increased exponentially with total length and linearly with total weight. Spawning frequency, estimated from the incidence of gravid females during the spawning period, was once every 3.4 days. This corresponds to an average of 38 spawns during the spawning period, and a total annual fecundity of 108,148 ova for a 116 mm fish to 641,478 ova for a 175 mm fish.

Blackcheek tonguefish have a relatively high mortality rate, small asymptotic length, high growth parameter K, and high L_{50}/L_{max}. This species also reproduces at a younger age and smaller size, and has a higher total fecundity than other Pleuronectiforms in this system. Blackcheek tonguefish have life history parameters that lie on the r-end of the r and K continuum. Species with r-selected life history parameters are hypothesized to be better adapted at exploiting seasonally dynamic and highly unpredictable environments such as Chesapeake Bay.

Age, growth, and reproductive biology of blackcheek tonguefish, *Symphurus plagiusa* (Cynoglossidae: Pleuronectiformes), in Chesapeake Bay, Virginia.

GENERAL INTRODUCTION

The blackcheek tonguefish (*Symphurus plagiusa*) is the only member of the pleuronectiform family Cynoglossidae occurring in Chesapeake Bay (Ginsburg 1951; Musick 1972; Munroe 1987). This shallow-water flatfish ranges from Connecticut southward through the Florida Keys, northern Gulf of Mexico, Cuba, and Bahamas (Munroe 1987; Topp and Hoff 1972). From Chesapeake Bay and southward it is an abundant component of the fish fauna in estuaries and coastal embayments. Throughout most of its range, blackcheek tonguefish occur in sufficient numbers to comprise a minor inclusion in several industrial fisheries, though it is usually considered a nuisance (Topp and Hoff 1972). In Chesapeake Bay, *Symphurus plagiusa* is the second most abundant pleuronectiform next to the hogchoker, *Trinectes maculatus* (Bonzek et al. 1992). *Symphurus plagiusa*, *Trinectes maculatus*, and the smallmouth flounder, *Etropus microstomus*, may be the only flatfishes completing their life cycles in Chesapeake Bay and its tributaries. Due to the relatively small size of these fishes, they are inaccessible to most fishing gears used in Chesapeake Bay and elsewhere.

The proposed research is significant for several reasons. Life history aspects of the blackcheek tonguefish, including growth, maturity, and reproduction, have yet to be studied rigorously. Despite its abundance in estuarine and marine habitats of the

northwest Atlantic, information on life history parameters of this species is limited, probably due to its low commercial value. Previous works have described recruitment (Miller et al. 1991), size-related maturity patterns (Munroe 1987), distribution, relative abundance and length frequencies (Shealy et al. 1974), and larval forms (Olney and Grant 1976; Hildebrand and Cable 1930); however, results by Hildebrand and Cable were compromised because at least two different species of *Symphurus* were included in their samples (Olney and Grant 1976). The proposed research outlines an investigation that will serve to increase the scientific knowledge of the life history parameters of this species and provide the basis for comparative work on congeneric species of symphurine tonguefishes, as well as other flatfish species. No published studies detailing age, growth, and reproductive biology for any of the 74 species in this genus are available.

Mean catch per haul of this species has declined for the Chesapeake Bay and the three Virginia tributaries from 1991 through 1994. Since this fish is not harvested recreationally or commercially, then recent changes in abundance are not directly due to fishing activities, but rather reflect some other change in the environment. Such changes could include a degradation of environmental quality of the Chesapeake Bay system, a change in recruitment patterns of this species to Chesapeake Bay, or some other aspect such as a change in trophic dynamics. Since this fish may spend its entire lifetime in Chesapeake Bay, the blackcheek tonguefish may prove to be a useful bioindicator of water and sediment quality of Chesapeake Bay.

The blackcheek tonguefish is unique among other members of the family Cynoglossidae in that it is the only species in the family that inhabits estuarine

environments in the seasonally dynamic region in north temperate latitudes. Study of the life history aspects of this species is necessary to provide the basis for comparison of what features of the life history make this species so unique among the cynoglossids.

CHAPTER 1

Age, growth, and mortality of blackcheek tonguefish, *Symphurus plagiusa*, in Chesapeake Bay, Virginia

INTRODUCTION

The blackcheek tonguefish, *Symphurus plagiusa* (Linnaeus 1766), ranges from Connecticut southward through the Florida Keys, northern Gulf of Mexico, Cuba, and Bahamas (Ginsburg 1951; Topp and Hoff 1972; Munroe 1987). From Chesapeake Bay and southward it is an abundant component of the fish fauna occurring in estuaries and coastal embayments. South of Chesapeake Bay, blackcheek tonguefish occur in sufficient numbers to form a component of bycatch of several fisheries, constitute a minor inclusion in several industrial fisheries, and shrimp trawlers often find it a nuisance, as specimens frequently become embedded in the webbing of trawls to such an extent as to interfere with the gear's effectiveness (Topp and Hoff 1972).

Chesapeake Bay represents the northernmost location of a major population for this species (Munroe 1987). The blackcheek tonguefish is the only member of the pleuronectiform family Cynoglossidae occurring in Chesapeake Bay (Ginsburg 1951; Musick and Wiley 1972; Munroe 1987). This species is the second most abundant pleuronectiform occurring throughout lower Chesapeake Bay and its tributaries. Only the hogchoker, *Trinectes maculatus*, is more commonly taken in bottom trawls of Chesapeake Bay (Bonzek et al. 1993). Occurrence of all life history stages in Chesapeake Bay is indicative that this is a resident species in this system (Olney and Grant 1976).

Despite its abundance in different estuarine and marine habitats in the northwest Atlantic, life history parameters of *S. plagiusa* are largely unknown. Some previous works have described distribution, relative abundance and length frequencies for blackcheek tonguefish in South Carolina estuaries (Shealy et al. 1974), summarized size-related maturity patterns for individuals throughout the entire range of the species (Munroe 1987), investigated factors influencing recruitment (Miller et al. 1991), and described daily growth rates of juveniles in Georgia estuaries (Reichert and van der Veer 1991). The minimal amount of published information on this species may in part be due to the relatively small size of the fish; it reaches a maximum size of about 210 mm total length (TL), but is most commonly taken by otter trawl at sizes ranging from 135 to 165 mm TL (Terwilliger 1996). Small size renders this species inaccessible to most recreational and commercial fishing gears used in Chesapeake Bay.

Few age and growth studies using bony structures exist for species of Cynoglossidae. Several studies have described growth for members of *Cynoglossus* from the eastern Atlantic (Chauvet 1971), western Pacific (Lin 1982; Meng & Ren 1988; Zhu & Ma 1992), and Indian Ocean localities (Seshappa 1974, 1978, 1981; Ramanathan et al. 1980; Seshappa and Chakrapani 1984). Previous work describing the age structure of blackcheek tonguefish in Chesapeake Bay used length frequencies of fish taken by otter trawl (Bonzek et al. 1993), but these results are limited because this type of analysis requires subjective interpretation of modal frequencies in the data, which, given the difficulty of distinguishing modal groups at older ages, renders the interpretation unreliable (White and Chittenden 1977; Jearld 1983; Barbieri 1993). No growth studies using bony

structures have been performed on species of *Symphurus*. This relatively large gap in knowledge for over 150 species of cynoglossid tonguefishes has recently been noted in a compilation of flatfish life history parameters (Miller et al. 1991).

Symphurus plagiusa is ideal for life history studies because it is unique among the Cynoglossidae in that it is the only species in the family that inhabits estuarine environments in the seasonally dynamic region of north temperate latitudes. Most cynoglossid species occur in the tropics and are difficult to age using bony elements because environmental constancy precludes the formation of interpretable growth marks on these structures. Those species of cynoglossid tonguefishes which occur in temperate waters generally are small sized, deep water forms which are difficult to catch in abundance.

This study was undertaken to determine age structure, longevity, and mortality for a population of *S. plagiusa* residing in Chesapeake Bay. Knowing age structure, growth rate, and longevity of this species provides a window of understanding into the biology of the Cynoglossidae, as well as provides insights into age, growth, and longevity of other small sized, estuarine dependent flatfishes.

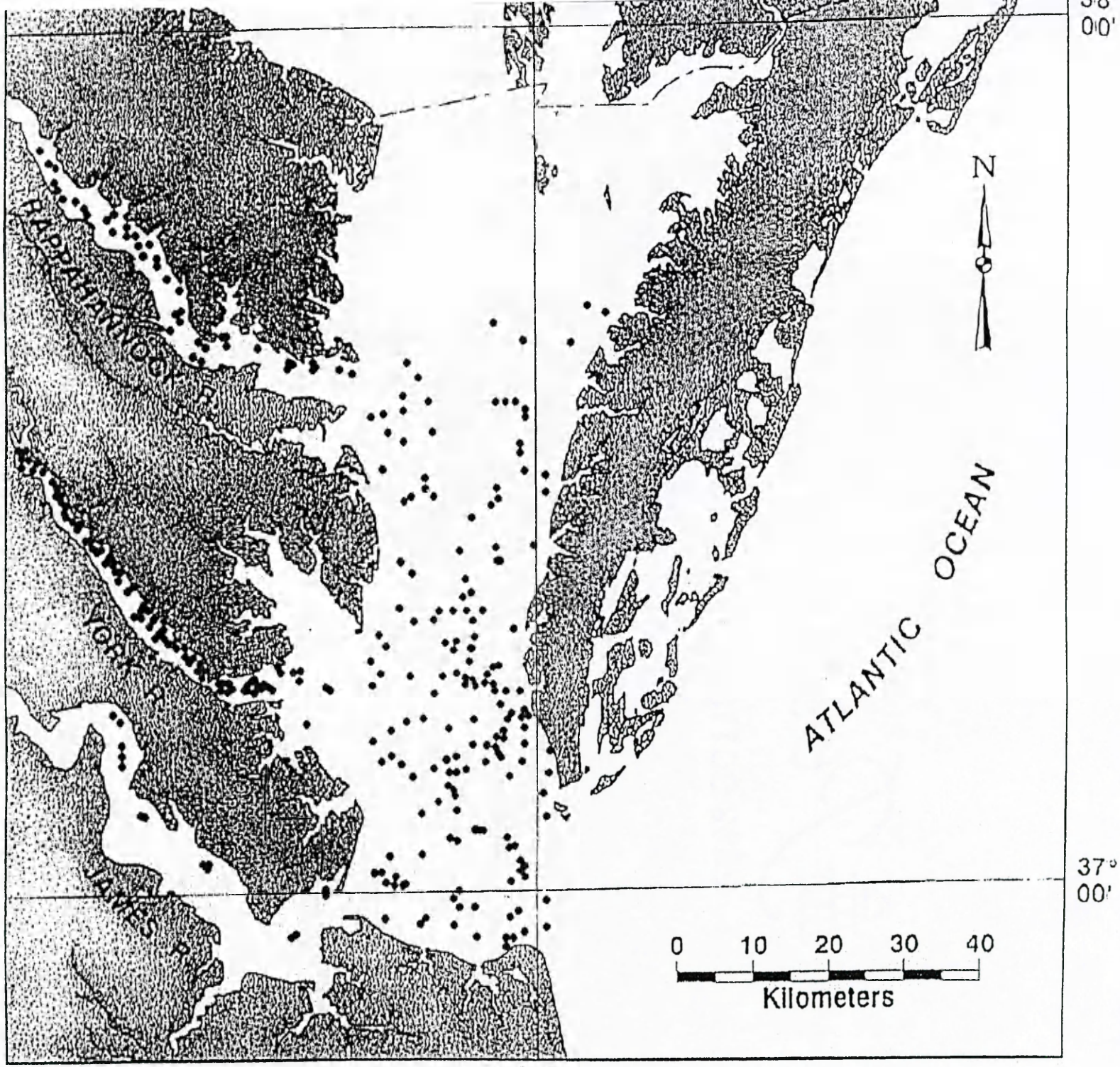
MATERIALS AND METHODS

Blackcheek tonguefish were collected primarily by 9.14 meter otter trawl with a 3.81 mm stretch mesh body, 0.64 mm mesh cod end liner, and attached tickler chain (Bonzek et al. 1993). Fish were collected from April 1994 through August 1995 primarily via the Virginia Institute of Marine Science (VIMS) juvenile finfish and blue crab stock assessment programs trawl survey. The survey employs a monthly, random stratified design of the lower Chesapeake Bay and fixed station mid-channel transects in each of the three major Virginia tributaries: the York, James, and Rappahannock rivers (Fig. 1). Details of sampling design are provided in Geer et al. (1994).

Samples of blackcheek tonguefish were randomly selected from trawl catches, brought to the lab, measured for total length (TL) to the nearest millimeter, and total weight (TW) taken to the nearest hundredth of a gram. Regressions were fit to the length-weight data, and regressions on log transformed data for male and female blackcheek tonguefish compared using analysis of covariance (ANCOVA).

A scale sample from a region in the posterior third of the body, dorsal to the midline, was taken from all fish. Six scales from each fish were cleaned with hydrogen peroxide and mounted on plastic strips using a Carver hydraulic laboratory press. A random sample of scale strips from 50 fish were then viewed with a microfiche reader in attempts to

Figure 1. The lower Chesapeake Bay system, including James, York, and Rappahannock rivers. Points indicate where blackcheek tonguefish, *Symphurus plagiusa*, were caught, April 1994-August 1995.


38° 00'
N
ATLANTIC OCEAN
37° 00'
0 10 20 30 40
Kilometers
76° 00'

discern annuli for comparison with otolith sections.

Both sagittal otoliths were removed, cleaned, and stored dry for later processing. Otolith maximum diameter was measured to the nearest 0.00001 mm from the anterior tip of the rostrum to the postrostrum using an Optical Pattern Recognition System by Biosonics. Sagittal otoliths randomly selected from each fish for age analysis were embedded in epoxy resin (Spurr, 1969) and sectioned transversely (1 mm thick) through the core. Sections were made using a Buehler Isomet low speed saw with duel diamond blades. Sections were mounted on glass slides using Crystal Bond adhesive, sanded using 1000 grit sandpaper to remove saw marks and to gain proximity to the core, polished with alumina powder, and examined with a binocular dissecting microscope (30x) using transmitted light and bright field.

Otolith annuli were validated by the marginal increment method (Bagenal and Tesh 1978). Distances from the core to each annulus and the proximal edge were computed by drawing a vertical line from the core to the proximal edge using an optical pattern recognition system (OPRS). Attention was paid to the width of the translucent margin between the last annulus and the proximal edge.

Regression analyses of otolith radius on total length and weight on total length were calculated by the method of least squares. Back-calculated lengths-at-age were computed using the Lee method (Lagler 1956):

$$L' = C+S'(L-C)/S$$

where L' = total length of the fish at time of annulus formation

L = total length of fish at time of capture

S' = measurement to the annulus

S = otolith radius at time of capture

C = correction factor; y-axis intercept of the regression of total length on otolith radius

To evaluate growth, back-calculated lengths-at-ages were fit to the von Bertalanffy model (Ricker 1975) using nonlinear regression (Marquardt method) via Fishparm (Saila et al. 1988). Likelihood-ratio tests were used to compare parameter estimates for males and females (Kimura 1980; Cerrato 1990).

Instantaneous total annual mortality rates, Z, were estimated from maximum age using Hoenig's pooled regression equation (Hoenig 1983), by calculating a theoretical total mortality for the entire lifespan following the reasoning of Royce (1972) as described in Chittenden and McEachran (1976), and by the regression method using a catch curve of trawl-survey data for all recruited ages. Mortality estimates were based on the linear, right hand portion of the curve, which is comprised of fish ages 3-5. Values of Z were converted to total annual mortality rates, A, using the relationship $A = 1\text{-}e^{-Z}$ (Ricker 1975).

RESULTS

A total of 566 fish were used for this study, encompassing a size range from 36 to 202 mm TL. This size range encompassed all individuals from post-settling juveniles to mature adults. Sex ratios for blackcheek tonguefish collected in this study were 223 males:343 females, or 1:1.54. Chi square analysis of sex ratios for 566 blackcheek tonguefish indicated statistically significant deviations ($\chi^2 = 25.44$; df=1) from a 1:1 sex ratio (Table 1). G-test of sex ratios for blackcheek tonguefish broken down into 25 mm length increment shows females dominated many intermediate size classes (Table 2).

The length-weight relationships for 200 males and 310 females were (Fig. 2):

Males: $W = 10^{-5.30} (TL^{3.126})$

Females: $W = 10^{-5.37} (TL^{3.163})$

No statistically significant differences between sexes in the total length-total weight relationships were found (ANCOVA; F=3.86; df=506; P>0.25). The length-weight relationship for sexes combined was:

$$W = 10^{-5.34} (TL^{3.148})$$

TABLE 1

Chi square analysis of sex ratio for blackcheek tonguefish, *Symphurus plagiusa*, in Chesapeake Bay.

	MALES	FEMALES	n	X2	P
frequency	223	343	566		
expected	283	283		25.4417	* P0.001=10.83

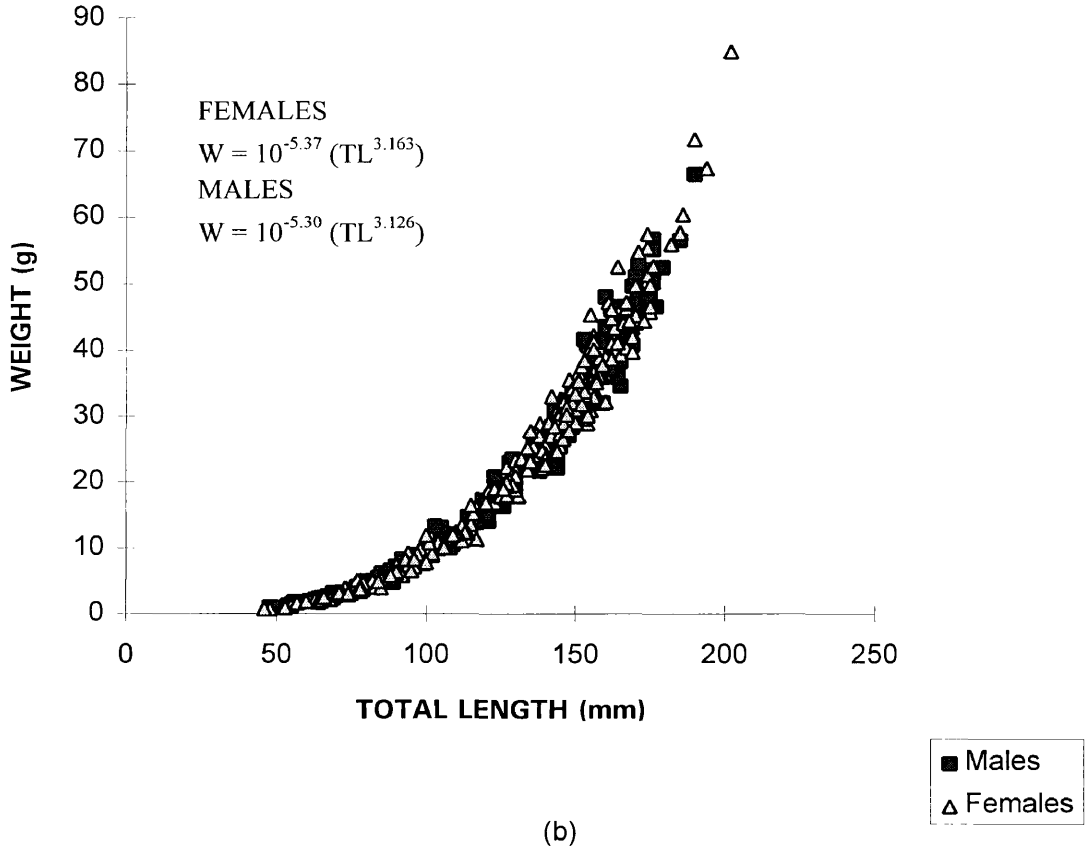
TABLE 2

G-test of sex ratios for blackcheek tonguefish, *Symphurus plagiusa*, from Chesapeake Bay: broken down by 25mm length increment.

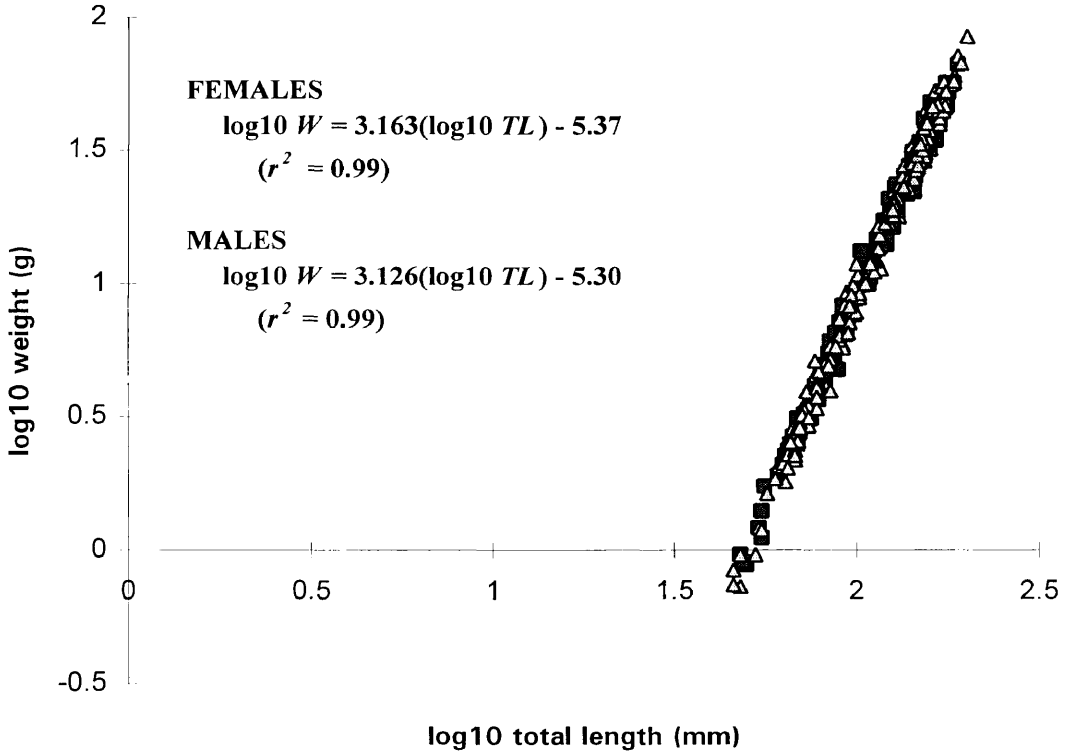
LENGTH (mm)	MALES	FEMALES	n	df	G	P
26-50	7.5	4.5	12	1	0.758015	ns
f(x)	6	6				
51-75	18.5	36.5	55	1	6.000851	P0.025=5.024
f(x)	27.5	27.5				
76-100	46.5	53.5	100	1	0.490401	ns
f(x)	50	50				
101-125	26.5	44.5	71	1	4.613566	P0.05=3.841
f(x)	35.5	35.5				
126-150	68.5	106.5	175	1	8.317527	P0.005=7.879
f(x)	87.5	87.5				
151-175	51.5	91.5	143	1	11.33949	P0.001=10.828
f(x)	71.5	71.5				
176-200	7.5	8.5	16	1	0.062541	ns
f(x)	8	8				
201-225	0.5	1.5	2	1	0.523248	ns
f(x)	1	1				
total	227	347	574	total 8	32.10564	P0.001=26.124
f(x)	287	287		pooled 1	25.27317	P0.001=10.828
				heterogeneity 7	6.8325	ns

Figure 2. Total length-weight relationship for both sexes of blackcheek tonguefish, *Symphurus plagiusa*, from Chesapeake Bay: (a) raw data; (b) log transformed data.

(a)


FEMALES
W = 10^-5.37 (TL^3.163)
MALES
W = 10^-5.30 (TL^3.126)
WEIGHT (g)
TOTAL LENGTH (mm)
0
10
20
30
40
50
60
70
80
90
0
50
100
150
200
250
Males
Females

(b)


FEMALES
log10 W = 3.163(log10 TL) - 5.37
(r^2 = 0.99)
MALES
log10 W = 3.126(log10 TL) - 5.30
(r^2 = 0.99)
log10 weight (g)
log10 total length (mm)
-0.5
0
0.5
1
1.5
2
2.5

Size at sexual maturity observed in female and male blackcheek tonguefish ranged between 80 and 130 mm TL and 70-110 mm TL respectively. Length at which 50% of the population reached maturity was 101 mm TL for females and 91 mm TL for males (Fig. 3).

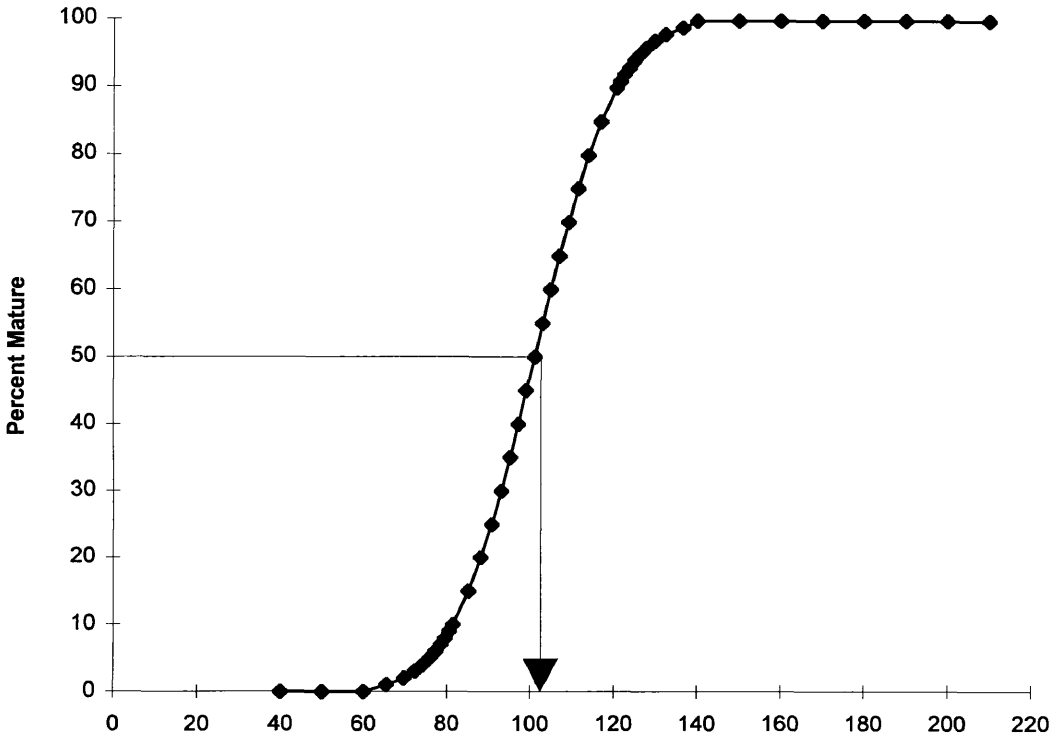
Scales proved to be an unreliable tool for ageing. Annuli were poorly defined and difficult to distinguish, and in many cases, the scales were unreadable. Fish ages obtained from scales agreed with ages obtained from otoliths in only 12% of the cases. This is not surprising, as scale annuli from known-age fish have been shown to be inconsistent indicators of age, even in relatively young fish (Prather 1967; Heidinger and Clodfelter 1987).

Sagittal otoliths from blackcheek tonguefish are small, round, dense structures that cannot be read whole. Transverse otolith sections exhibit fairly distinct, opaque bands that can be used for ageing. Sections showed that the otoliths have an opaque core surrounded by a wide opaque area. The outer edge of this opaque area represents the first annulus. Size and appearance of this first annulus varies greatly among fish, from a broad band continuous with the core, to a narrow opaque band not continuous with the core. Subsequent annuli are represented by thin opaque bands that encircle the core. Broader translucent zones were found between annuli. The combination of a broad translucent zone and a fine opaque zone represents one year's growth (Fig. 4).

Annuli were counted on otoliths from 566 blackcheek tonguefish measuring 36-202 mm TL. In 185 (32.7%) cases, the first and second readings did not agree and a third reading had to be made. The majority of disagreements occurred during the beginning of

Figure 3. Length at maturity for blackcheek tonguefish, *Symphurus plagiusa*, in Chesapeake Bay: (a) females; (b) males. Arrow represents length at 50% maturity.

(a) Females



(b) Males

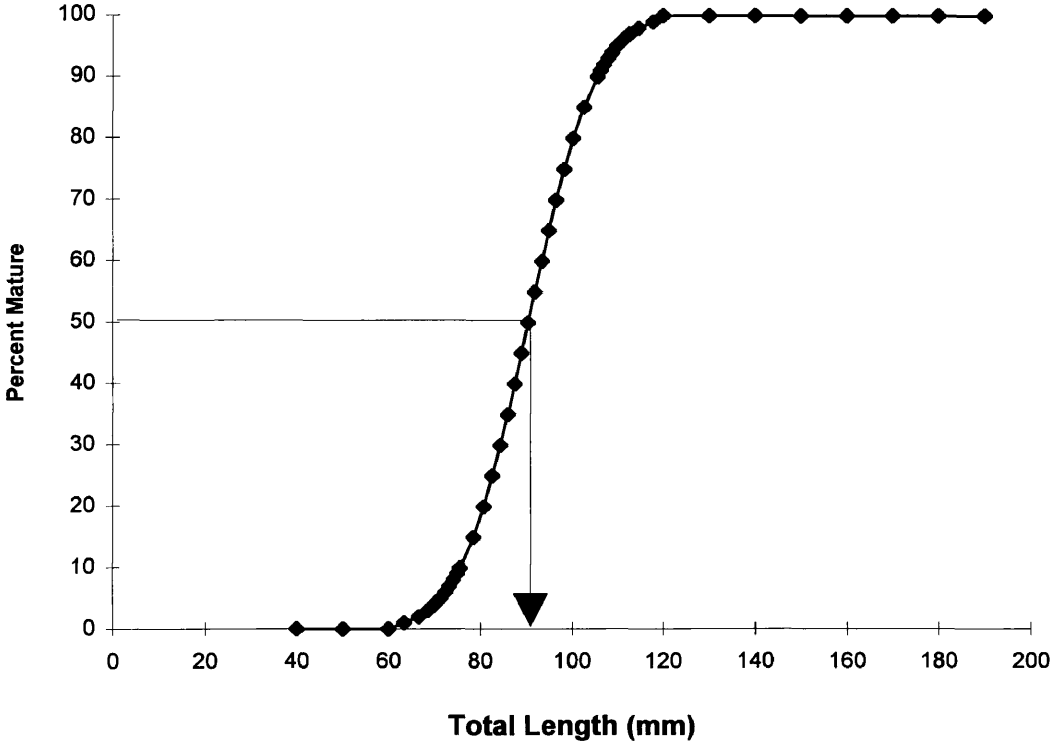
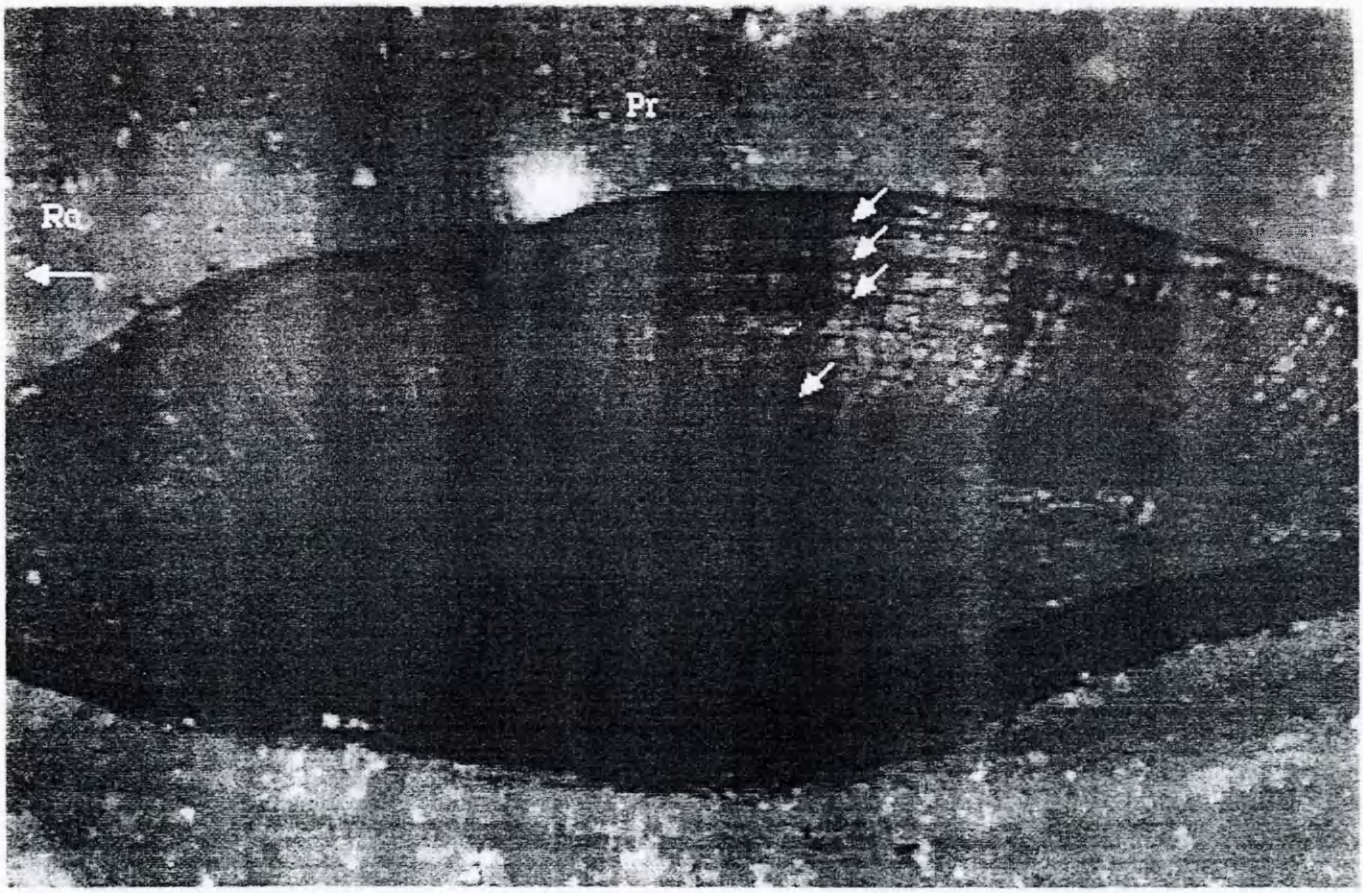


Figure 4. Transverse otolith section from a blackcheek tonguefish, *Symphurus plagiusa*, collected in Chesapeake Bay. Age 4+. Arrows indicate annuli. Pr = proximal; Ro = rostral.


Pr
Ra

the study; subsequent re-ageing resolved most discrepancies in age estimates. In most cases, the second reading differed only by one year. In 7 (1.24%) cases, the third reading was different than the first and second. These otoliths were considered unreadable and excluded from further analysis.

Otolith maximum diameter (*OD*) was linearly related to total length (Fig. 5):

$$TL = 64.6(OD)\text{-}17.0 \qquad (r^2 = 0.94)$$

This linear relationship is indicative that otolith growth is proportional to fish growth.

Greatest incremental growth in TL for both sexes occurred during the first year and then declined. The mean back-calculated length for females and males after their first year was 78.84 mm and 75.67 mm, respectively. Growth for both sexes in the second year was only 39-41% of that recorded from the first year. Growth increments continued to decrease every year for both sexes until age 5, when the growth increment increased slightly for both sexes. This increase at age 5 may be due to the relatively small number of samples of this age group for both sexes.

Monthly mean marginal increments were plotted for all age groups combined (Fig. 6). The seasonal progression of marginal increment was similar for all age groups. Annuli were closest to the margin in June. Marginal increments peaked in August, then decreased and leveled off from September through May. Monthly mean marginal increment plots show only one trough during the year, indicating that only one annulus is formed yearly.

Males and females appear to have similar lengths-at-age until age 4. After age 4, mean lengths for females were slightly longer than for males. The largest male collected in this

Figure 5. Linear regression of total length (mm) on otolith maximum diameter (mm) for blackcheek tonguefish, *Symphurus plagiusa,* from Chesapeake Bay.

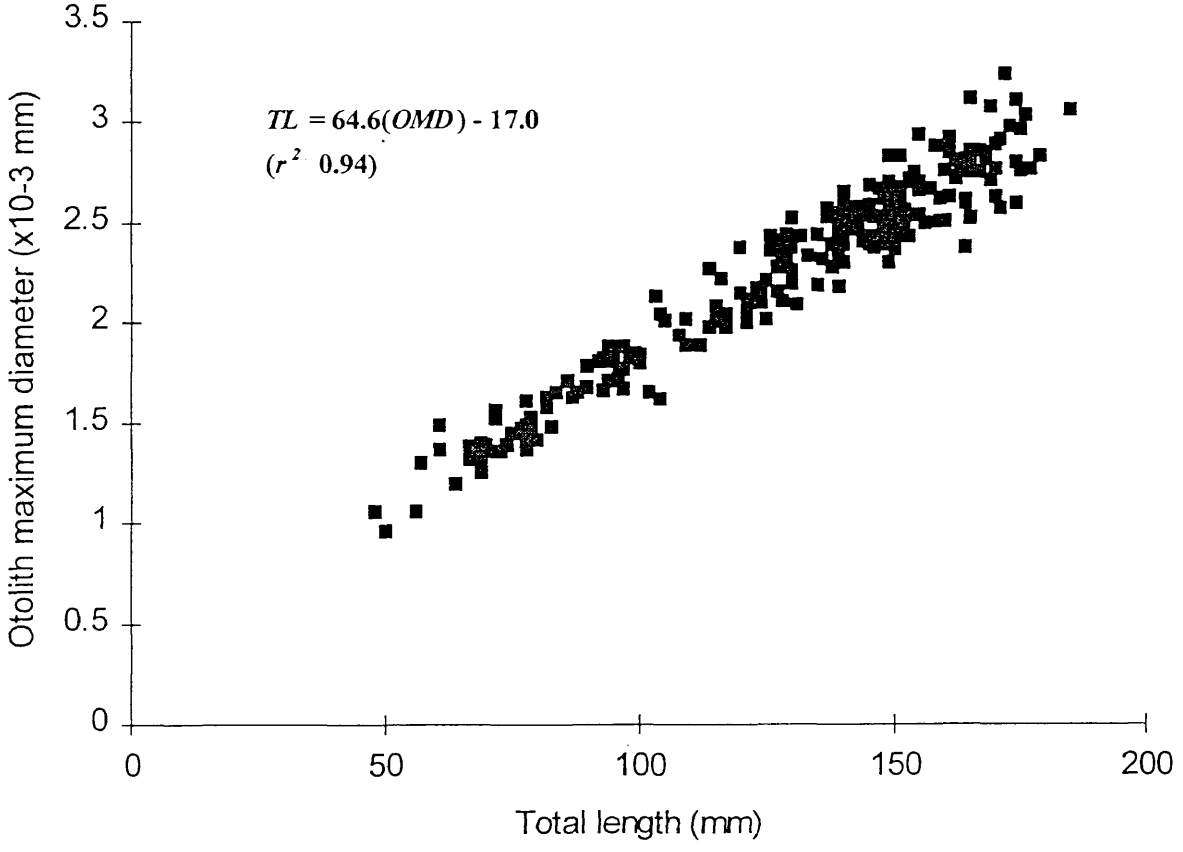
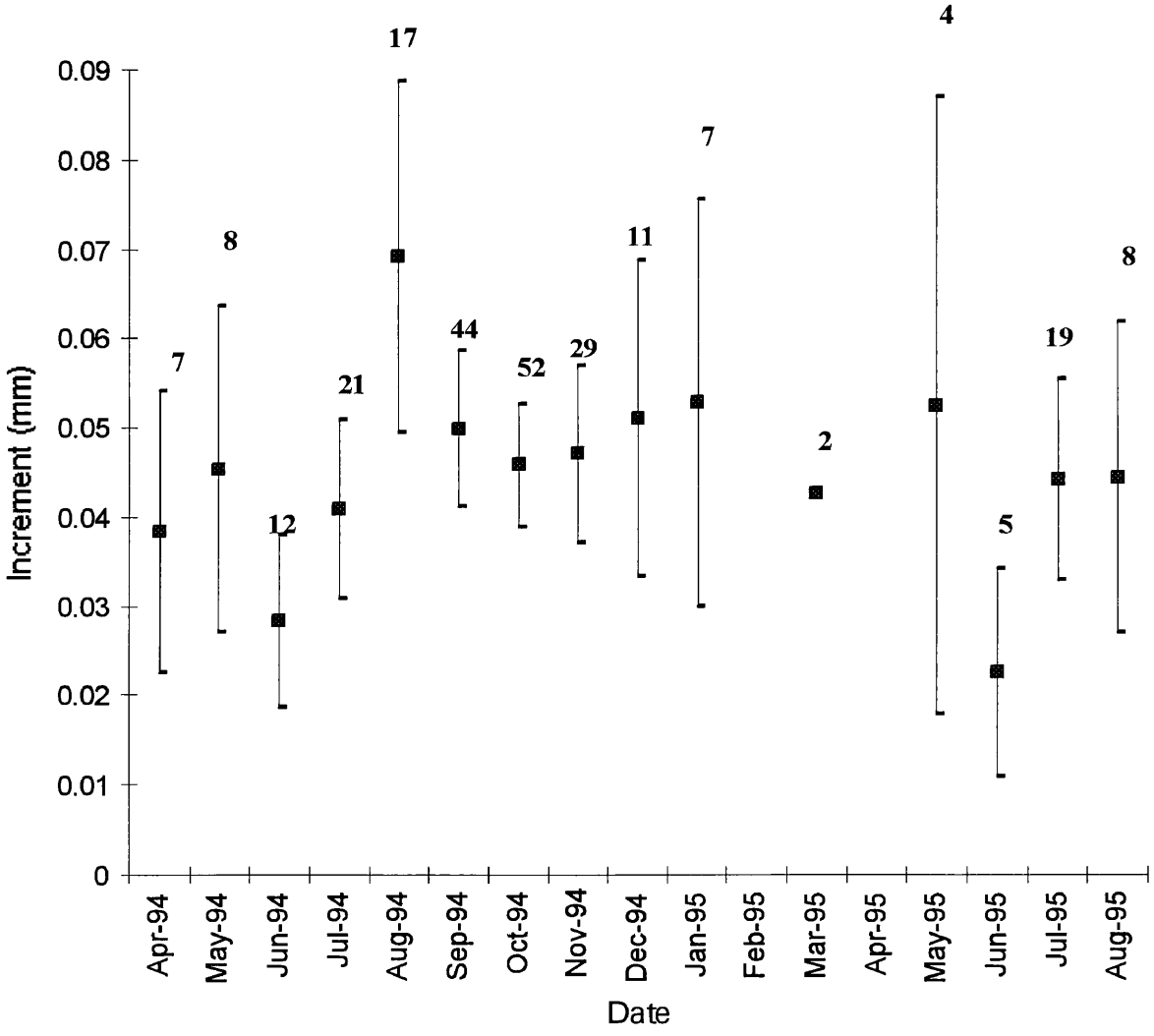

3.5
3
2.5
2
1.5
1
0.5
0
Otolith maximum diameter (x10-3 mm)
TL = 64.6(OMD) - 17.0
(r² 0.94)
0
50
100
150
200
Total length (mm)

Figure 6. Plot of monthly mean marginal increment for blackcheek tonguefish, *Symphurus plagiusa*, ages 1-5. Vertical bars represent ± 1 standard error. Numbers above bars represent monthly sample sizes.


Increment (mm)
0.09
0.08
0.07
0.06
0.05
0.04
0.03
0.02
0.01
0
7
8
12
21
17
44
52
29
11
7
2
4
5
19
8
Apr-94
May-94
Jun-94
Jul-94
Aug-94
Sep-94
Oct-94
Nov-94
Dec-94
Jan-95
Feb-95
Mar-95
Apr-95
May-95
Jun-95
Jul-95
Aug-95
Date

study was 190 mm (age 5+), while the largest female collected was 202 mm (age 5+).

Mean lengths at age were back-calculated for 92 males and 145 females (Table 3). Observed (empirical) lengths were consistently higher than the back-calculated lengths-at-age for individual age groups, which indicated seasonal growth had occurred since formation of a new annulus. Differences between back-calculated lengths-at-age and observed lengths are in the range of observed seasonal growth.

Von Bertalanffy parameters were computed, using lengths back-calculated to the most recent annuli to reduce bias in asymptotic length and growth coefficient due to the presence of Lee's phenomenon (Vaughn and Burton 1994). Lee's phenomenon was evident in the back-calculated lengths-at-age. Resulting parameters and equations for both sexes pooled and separate are as follows:

Sexes combined:

$K = .3081$

$L_\infty = 192.4$

$t_0 = -.8315$

$L_t = 192.4(1\text{-}e^{-.3081(t+.8315)})$

Males

$K = .2853$

$L_\infty = 196.5$

$t_0 = -.9195$

$L_t = 196.5(1\text{-}e^{-.2853(t+.9195)})$

TABLE 3

Mean observed and back-calculated lengths for female and male blackcheek tonguefish, *Symphurus plagiusa*, from Chesapeake Bay: ages 1-5.

FEMALES

Age	Sample Size	Mean Observed Length	Mean Back-Calculated Lengths at Successive Annuli I	II	III	IV	V
1	15	121.07	83.943				
2	31	136.81	81.156	115.272			
3	64	153.33	76.038	113.238	135.832		
4	25	161.62	70.073	101.826	126.549	145.385	
5	10	181.20	82.966	108.644	129.812	150.714	167.936
		Mean Length	78.835	109.745	130.731	148.049	167.936
		Growth Increment	78.835	30.910	20.986	17.318	19.887

MALES

Age	Sample Size	Mean observed Length	Mean Back-Calculated Lengths at Successive Annuli I	II	III	IV	V
1	12	115.83	81.778				
2	22	136.59	82.844	113.864			
3	31	154.19	76.573	110.408	133.553		
4	23	166.52	73.339	107.662	129.228	148.911	
5	4	177.75	63.838	95.125	118.058	141.277	163.721
		Mean length	75.675	106.765	126.946	145.094	163.721
		Growth increment	75.675	31.090	20.182	18.147	18.628

Females

$K = .3205$

$L_\infty = 190.6$

$t_0 = -.7842$

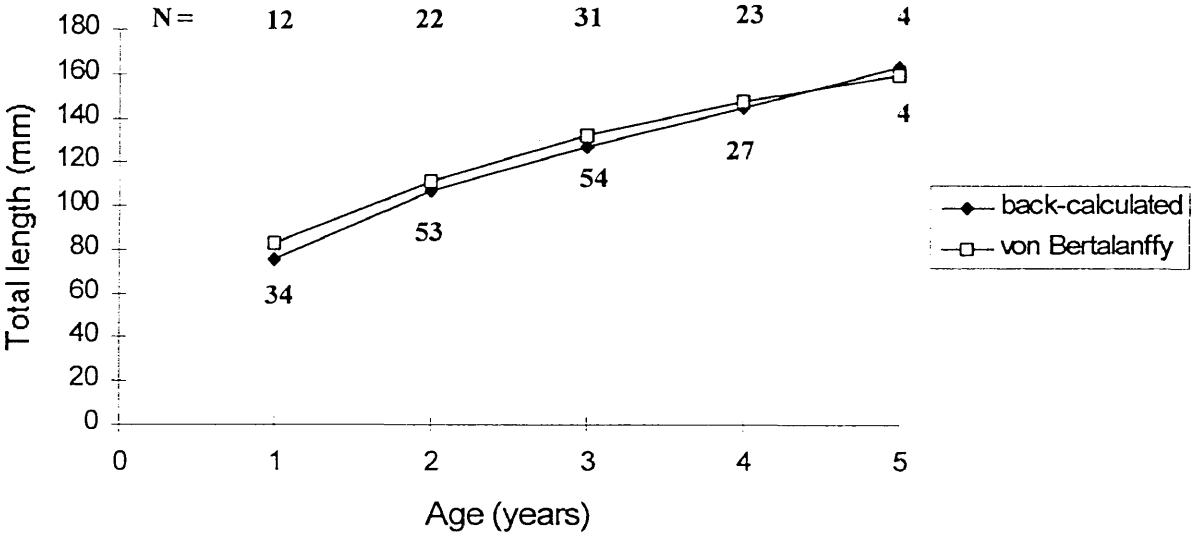
$L_t = 190.6(1\text{-}e^{-.3205(t+.7842)})$

K-value for female blackcheek tonguefish (.3205) was greater than that (.2853) calculated for males. Females were also larger in size (TL) for ages 1, 2, and 3. Males were slightly larger for ages 4 and 5. Males and females achieved 50% of L_∞ by the end of their first year and 75% between ages 2 and 3 (Fig. 7). Results of likelihood-ratio tests showed no significant difference in the overall von Bertalanffy growth models for males and females (χ^2=6.56, df=3, P=0.09). Estimates of L_∞ (χ^2=0.10, df=1, P=0.75), k (χ^2=0.03, df=1, P=0.87), and t_0 (χ^2=0.19, df=1, P=0.67) were not significantly different between sexes (Table 4).

Von Bertalanffy lengths compared favorably with back-calculated lengths-at-age for both sexes (Fig. 7). The maximum reported size for *S. plagiusa* is 210 mm TL (Wenner and Sedberry 1989). The largest male collected in this study was 190 mm and the calculated L_∞ for males was 196.5 mm. The largest female collected in this study was 202 mm and the calculated asymptotic length for females was 190.6 mm. This is expected because asymptotic length is a regression estimate, thus an average, that represents an average maximum length if fish live and grow according to the von Bertalanffy equation. The estimated asymptotic length value represents a reasonable average maximum length

Figure 7. Plot of back-calculated and von Bertalanffy lengths-at-age corrected for Lee's phenomenon for blackcheek tonguefish, *Symphurus plagiusa*, from Chesapeake Bay: (a) males; (b) females. Numbers above plots represent total number of fish at each age. Numbers below plots represent sample size of back-calculated lengths at each age.

(a)


N=
12
22
31
23
4
4
27
54
53
34
back-calculated
von Bertalanffy
Total length (mm)
Age (years)
0
20
40
60
80
100
120
140
160
180
0
1
2
3
4
5

(b)

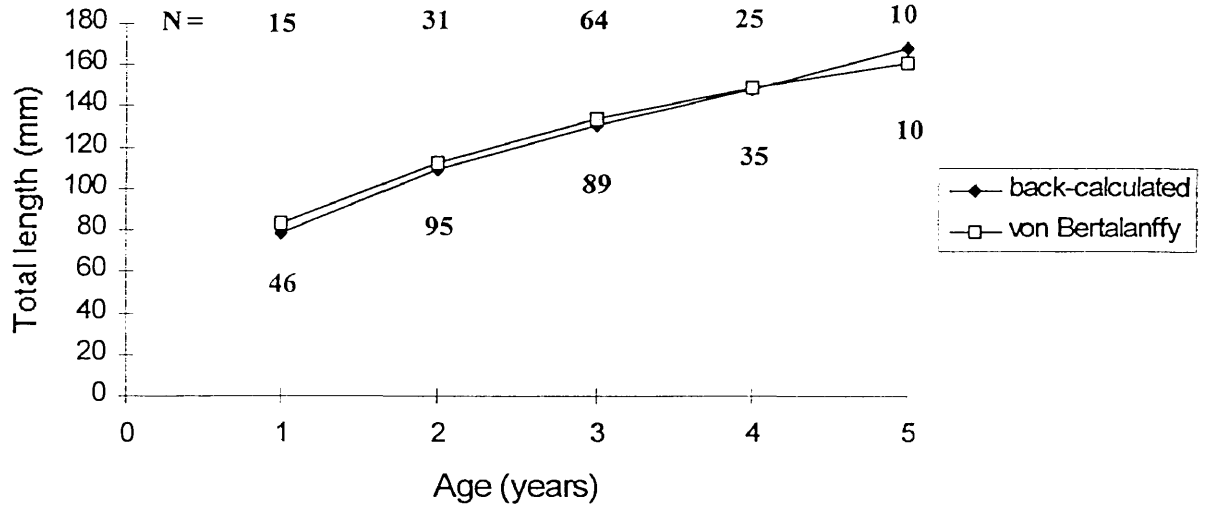

N=
15
31
64
25
10
10
35
89
95
46
back-calculated
von Bertalanffy
Total length (mm)
Age (years)
0
20
40
60
80
100
120
140
160
180
0
1
2
3
4
5

TABLE 4.

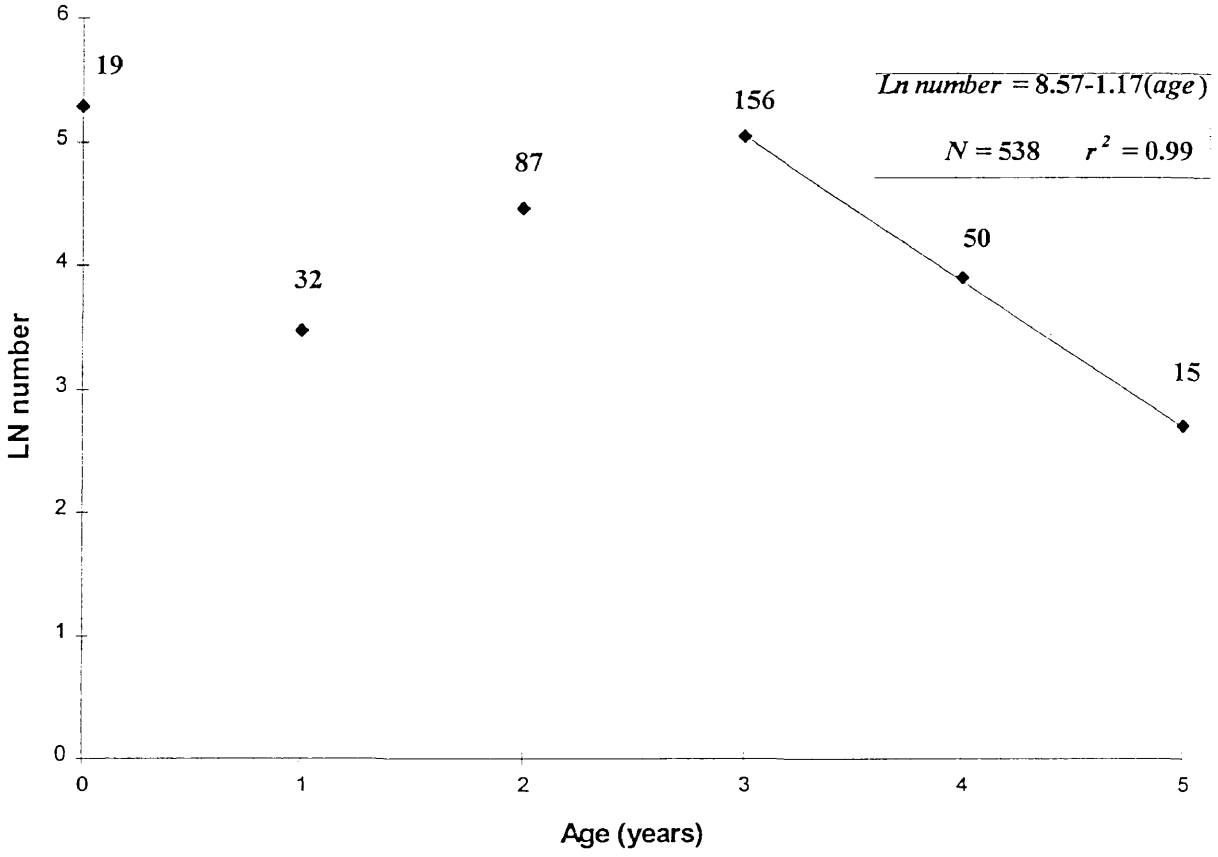
Likelihood ratio tests comparing von Bertalanffy parameter estimates for male (1) and female (2) blackcheek tonguefish, *Symphurus plagiusa* from Chesapeake Bay, Virginia. df = degrees of freedom

Hypothesis	Linear constraints	Residual SS	$\bar{x}_r^2$	df	P
HΩ	none	99,760.5			
Hω1	$L_{\infty 1} = L_{\infty 2}$	99,780.9	0.1043	1	0.7467
Hω2	$K_1 = K_2$	99,765.8	0.0271	1	0.8692
Hω3	$t_{01} = t_{02}$	99,796.9	0.1861	1	0.6661
Hω4	$L_{\infty 1} = L_{\infty 2}$ $K_1 = K_2$ $t_{01} = t_{02}$	10,1051.6	6.558	3	0.0874

estimate for the species.

Instantaneous total mortality (Z) ranged from 0.87 to 1.17. Estimates obtained for a maximum age of 5 years were 0.87 (A=58%) using Hoenig's (1983) method, and 0.92 (A=60%) using Royce's (1972) method. A regression estimate obtained from the slope of the right limb of the catch curve was 1.17 (A=69%) (Fig. 8).

Figure 8. Catch curve for blackcheek tonguefish, *Symphurus plagiusa*, collected by otter trawl in Chesapeake Bay, 1993-1995. Numbers above plot represent numbers at each age. Regression equation reflects the straight, right-hand limb of the curve.


Ln number = 8.57-1.17(age)
N = 538 r² = 0.99
19
32
87
156
50
15
LN number
Age (years)
0
1
2
3
4
5
6

DISCUSSION

This is the first detailed study presenting information on age, growth, and longevity for blackcheek tonguefish. Sagittal otoliths are valid tools for ageing *S. plagiusa*. They satisfy all of Van Oosten's (1929) criteria for ageing using hard parts. Otoliths are easy to locate and remove from fish, and are fairly easy to prepare and read. Marginal increment analysis shows that growth marks are formed once yearly, in June. Scales proved to be unsuitable for ageing this species, as only 12% agreement exists between ages obtained from scales and otoliths. These data show that only one growth mark is laid down each year, during early summer (June). Annuli are difficult to observe when they are at the margin of the otolith, until sufficient growth has occurred after annulus formation. Since ring formation occurs at the same time as the spawning season commences, annulus formation reflects allocation of energy resources to gonadal growth at the expense of somatic growth.

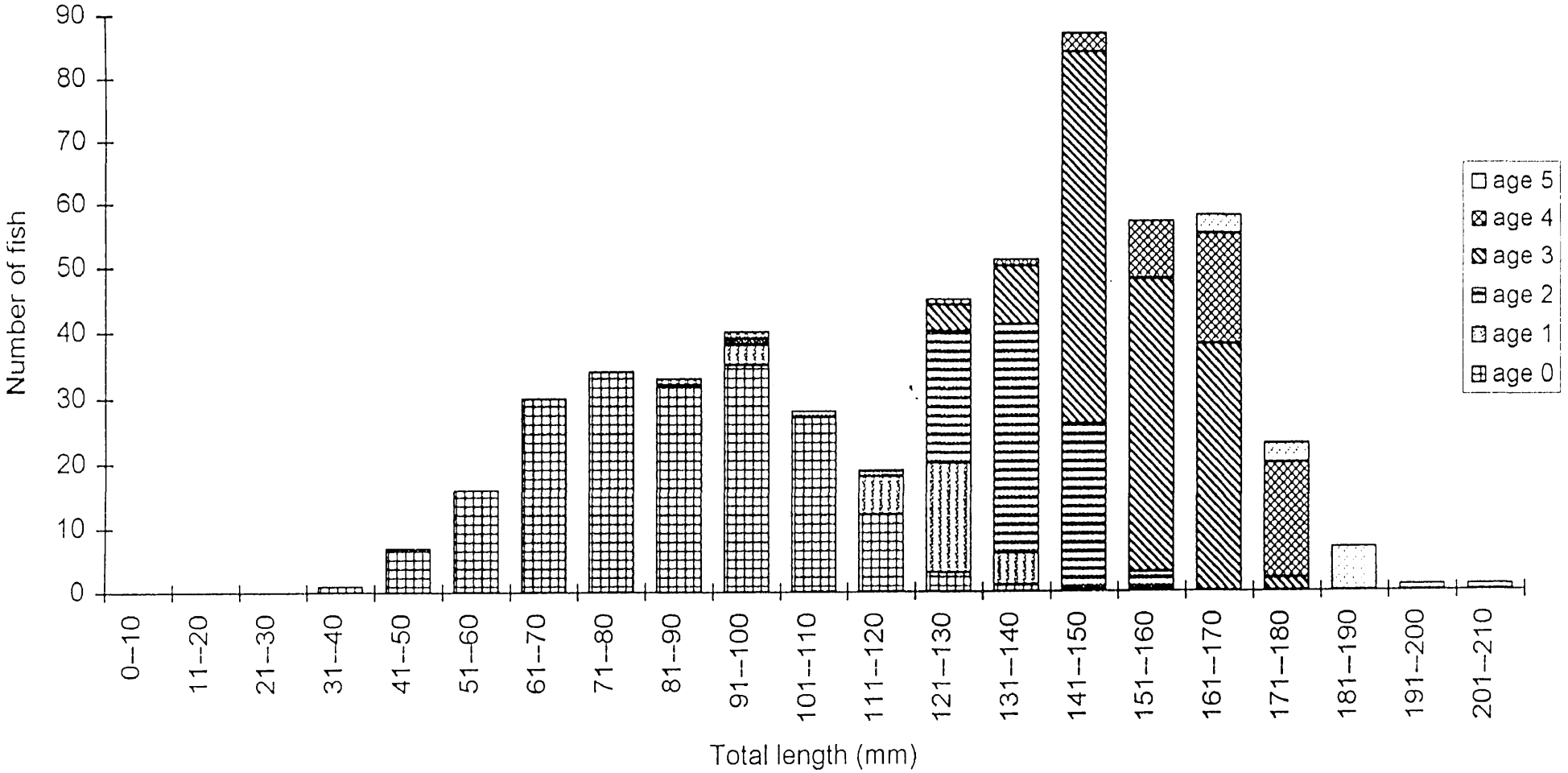
A commonly observed feature of flatfish is strong sexual dimorphism in size at age (Moyle and Cech 1988; Armstrong et al. 1992). Observed differences in back-calculated lengths between males and females beyond age 4 are not significant. Female blackcheek tonguefish grow at a slightly faster rate than do males, which is a commonly observed feature of flatfish (Chen et al. 1992; Santos 1994).

Back-calculated lengths-at-age were consistently less than observed lengths-at-age for all age groups combined. This may be attributed to Lee's phenomenon. Frequently the larger fish in a year-class have a greater mortality rate than smaller fish. This can be detected when back-calculations of length at earlier ages are made from hard parts, using samples that are representative of the whole of each age group involved. When a larger proportion of older fish die, the result is a smaller estimated size for fish at younger ages, than the true average size at the age in question (Ricker 1975; Gerking 1957). Since blackcheek tonguefish are not harvested commercially or recreationally, the presence of Lee's phenomenon in the back-calculations is due to differential mortality or possibly results from fish emigrating from the system. Larger and perhaps older individuals are known to occur on the continental shelf, where samples were not taken.

Length frequency data for this species show a bimodal distribution (Fig. 9), but this distribution does not adequately reflect the age composition of the individuals, indicating that length frequency analyses are unreliable as age estimators for this species. Six age groups are represented in the distribution. The high variability of observed lengths-at-age indicates that size is not a good predictor of age for blackcheek tonguefish. Much overlap exists in total length between age groups, especially in those of medium and large-sized fish.

Sex ratio deviated from 1:1 due to large single-sex catches of females in the lower Bay during the summer months. Hogchoker, a sympatric pleuronectiform species, was reported by Mansuetti and Pauly (1956) as displaying similar sexual separation in the

Figure 9. Length frequency by age of blackcheek tonguefish, *Symphurus plagiusa*, from Chesapeake Bay.


Number of fish
90
80
70
60
50
40
30
20
10
0
0–10
11–20
21–30
31–40
41–50
51–60
61–70
71–80
81–90
91–100
101–110
111–120
121–130
131–140
141–150
151–160
161–170
171–180
181–190
191–200
201–210
Total length (mm)
age 5
age 4
age 3
age 2
age 1
age 0

summer months prior to spawning, with females occupying grass bed and shoal regions. Dense concentrations of female blackcheek tonguefish were commonly found in deeper areas of lower Chesapeake Bay.

North temperate estuaries are rigorous physical systems well known for environmental extremes in temperature, salinity, and dissolved oxygen, and with strong seasonal cycles of primary and secondary production. Representatives of five different pleuronectiform families (Cynoglossidae, Achiridae, Paralichthyidae, Scophthalmidae, and Pleuronectidae) inhabit Chesapeake Bay. This assemblage includes representatives of cold temperate and warm temperate flatfishes. Chesapeake Bay occurs at the northern end of a warm temperate region and the southern end of a cold temperate region. The unique geographical location of Chesapeake Bay may account for the presence of both warm and cold water groups occurring there. The diverse families of pleuronectiforms inhabiting Chesapeake Bay makes for an interesting comparison of age and growth parameters of these fishes. Six species found in Chesapeake Bay include blackcheek tonguefish and hogchoker, which complete their life cycles in Chesapeake Bay, and summer flounder (*Paralichthys dentatus*), windowpane (*Scophthalmus aquosus*), winter flounder *(Pseudopleuronectes americanus*) and southern flounder (*Paralichthys lethostigma*), which do not complete their life cycles in Chesapeake Bay.

Few published age-growth studies for non-commercial Pleuronectiformes occurring in temperate estuaries of the western north Atlantic exist. The von Bertalanffy growth parameters for species other than blackcheek tonguefish were calculated using historical data found in the literature. These data show that blackcheek tonguefish have the highest

value for K (Table 5), and therefore reach asymptotic length faster than the other flatfishes (Francis 1996). Growth is fast in the first year then decreases thereafter. These fish mature at age 1. Beyond age 1, growth slows, at a time coincident with maturation. This slowing in somatic growth may reflect energy allocation diverted to gonadal development, or some other physiological process associated with maturation. In comparison, summer flounder mature at age 3, windowpane mature between ages 3&4, winter flounder mature at age 4 or 5, and hogchoker at age 2. Maturation at a later age allows for allocation of more energy towards somatic growth prior to the onset of maturation. This would account for the relatively rapid increase in size during early years of these fishes life histories (Fig. 10).

Asymptotic length also varies considerably among species. Asymptotic length for summer and southern flounder, (859 and 760 mm, respectively) greatly exceeds that for blackcheek tonguefish and hogchoker (192.4 and 209.5 mm, respectively). In fact, these fish grow more in their first year than blackcheek tonguefish and hogchokers do in a lifetime. Windowpane and winter flounder display asymptotic lengths in the midrange of this comparison of estuarine flatfishes.

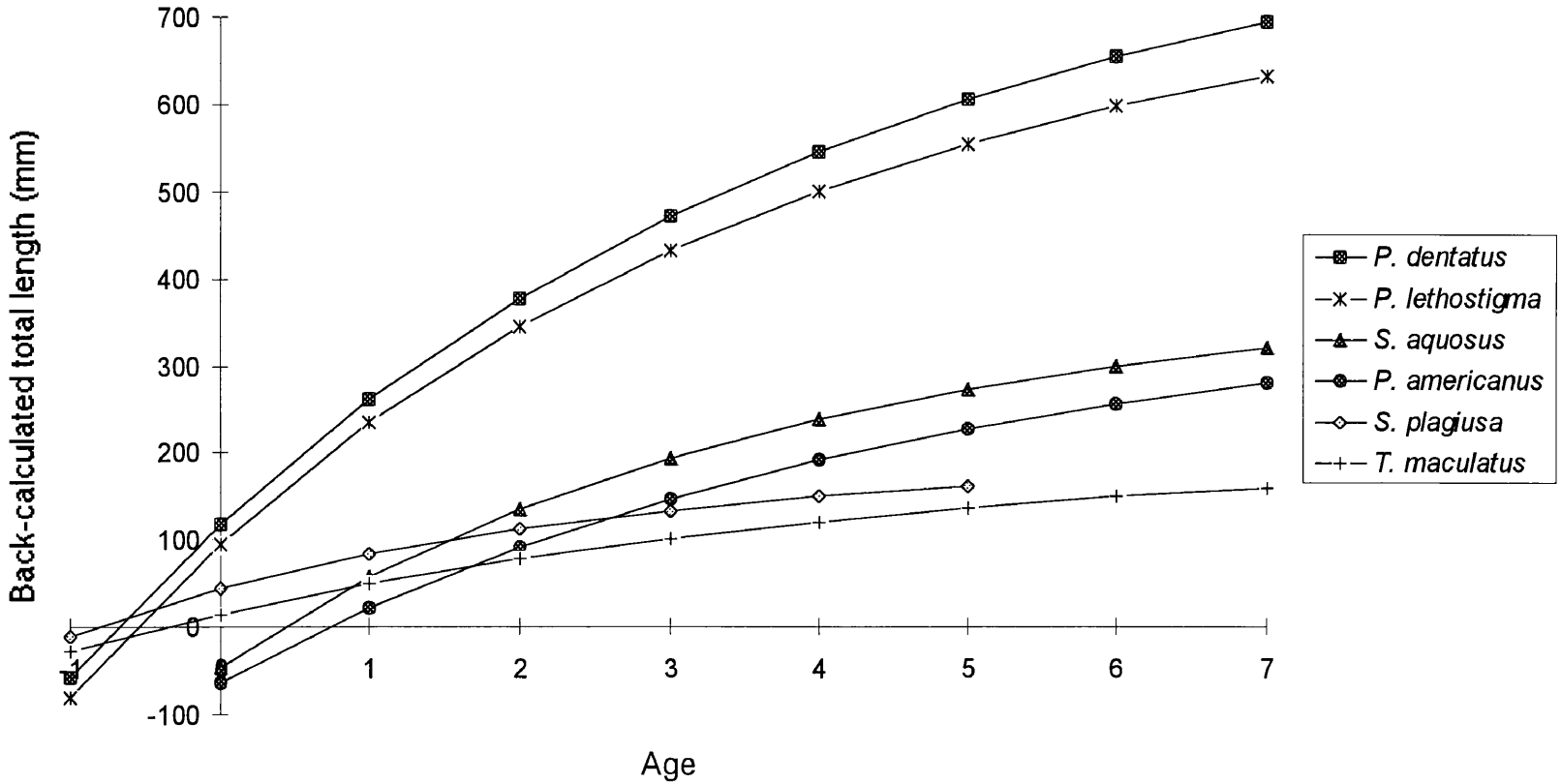
The representatives of these five different families of flatfishes display wide variety in growth parameters. Growth in fishes is regulated by genetic and environmental factors. The relationship among habitat, life-history strategies, and population parameters has been termed r and K selection (Adams 1980). The comparative nature of r and K selection is due to the fact that the continuum is an idealized model, and no species can be r or K selected in an absolute sense. Under an environmental regime with a large component of

TABLE 5

Comparison of von Bertalanffy parameters of selected Pleuronectiformes occurring in temperate estuaries of the western north Atlantic.

Species	Parameters	Source
Paralichthys dentatus (♀♂)	**K: .215** **L_∞: 859 mm** **t_0: -0.69**	**Desfosse 1995**
Paralichthys lethostigma (♀)	**K: .2346** **L_∞: 760 mm** **t_0: 0.5702**	**Miller et al. 1991**
Scophthalmus aquosus (♀)	**K: .272** **L_∞: 383 mm** **t_0: 0.418**	**Miller et al. 1991**
Pseudopleuronectes americanus (♀)	**K: .217** **L_∞: 375.8 mm** **t_0: 0.73**	**Miller et al. 1991**
Symphurus plagiusa (♀♂)	**K: .3081** **L_∞: 192.4 mm** **t_0: -0.8315**	**Terwilliger, this study**
Trinectes maculatus (♀)	**K: .195** **L_∞: 209.5 mm** **t_0: -0.3528**	**Miller et al. 1991**

Figure 10. von Bertalanffy growth curves for selected Pleuronectiformes occurring in western north Atlantic temperate estuaries. Values for species other than *Symphurus plagiusa* were derived from the literature.

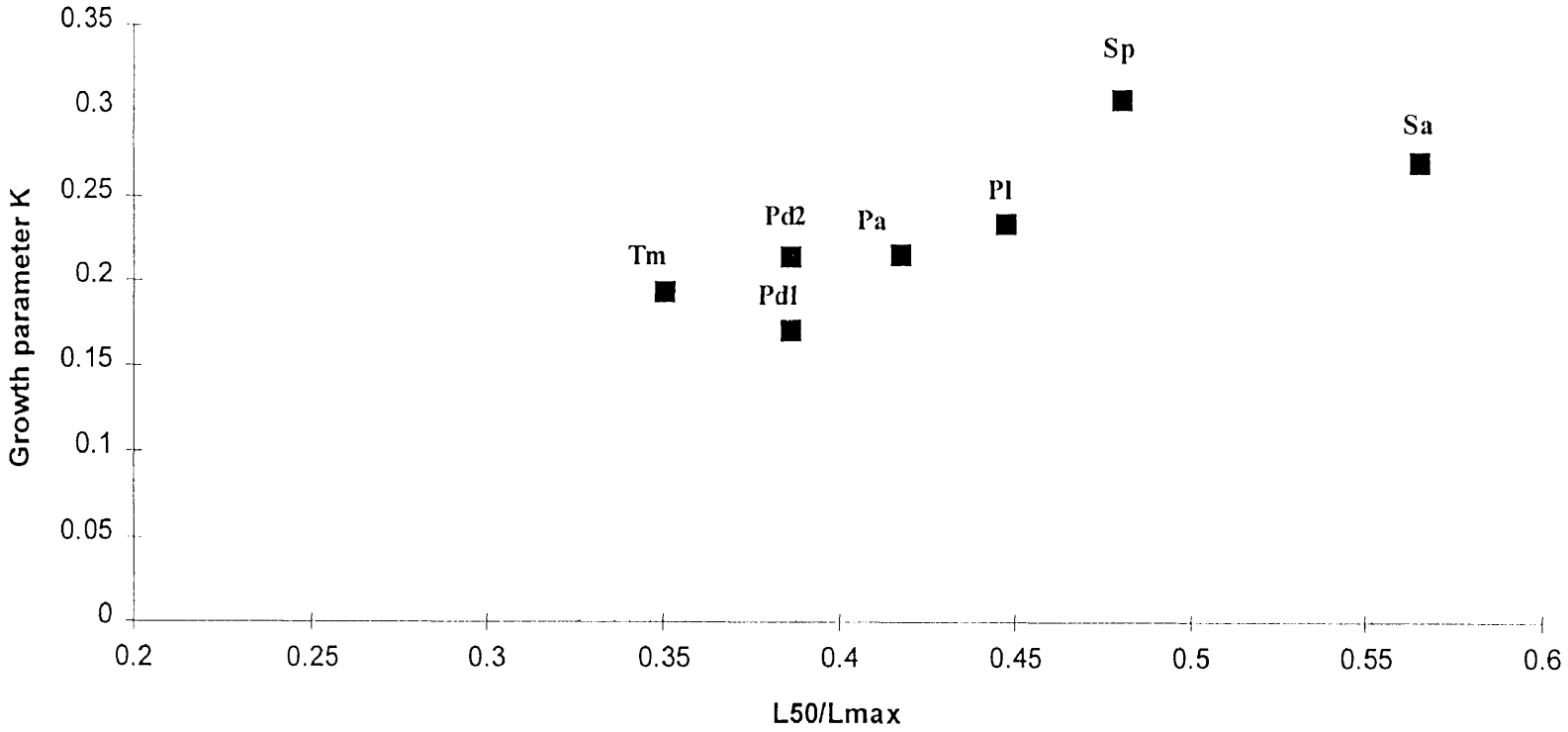

Back-calculated total length (mm)
700
600
500
400
300
200
100
0
-100
1
2
3
4
5
6
7
Age
P. dentatus
P. lethostigma
S. aquosus
P. americanus
S. plagiusa
T. maculatus

unpredictable, nonselective mortality, an organism will allocate more energy towards ensuring reproductive success. Early age at maturity, rapid growth rates, high virility, and shorter life span indicate *r*-selected features. *K*-selected features include later age at maturity, reduced growth rates, lower virility, and longer lifespan. These features, exhibited by organisms which experience predictive, selective mortality, increase individual fitness at the expense of reproductive activity (Adams 1980).

Miller et al. (1991), in an attempt to determine the degree of density dependence in stock recruitment, plotted growth parameter K vs. ratio of L_{50}/L_{max} (length at 50% maturity/total length) for several species of North American flatfish. This correlation represents a gradient from relatively *K*- to *r*- selected species of flatfishes. The addition of blackcheek tonguefish to Miller et al.'s graph (Fig. 11) shows that this species reaches L_{∞} rapidly, and has a high L_{50}/L_{max} ratio.

Blackcheek tonguefish exhibit relatively high instantaneous total mortality when compared with other northwestern Atlantic Pleuronectiformes (Table 6). Both Hoenig's and Royce's mortality estimates are based on the maximum known age of each species. In the comparison, blackcheek tonguefish are the shortest-lived species, and therefore have the highest instantaneous total mortality. Mortality estimates based on catch curve data reflect decreasing numbers of individuals in older age classes. Instantaneous total mortality of blackcheek tonguefish based on catch curve data is also greater than that for other Pleuronectiformes. Relatively few blackcheek tonguefish live to ages older than three years. Southern flounder, with a catch curve mortality estimate of 1.15, also has relatively few fish living to their maximum age. Hogchoker exhibits the smallest value of

Figure 11. Growth parameter *K* estimated from von Bertalanffy function plotted against the ratio of length at 50% maturity (L50) and maximum female length (Lmax). Abbreviations are as follows: *Tm* = *Trinectes maculatus*; *Pd1* = *Paralichthys dentatus* (Miller et al. 1991); *Pd2* = *Paralichthys dentatus* (Desfosse 1993); *Pa* = *Pseudopleuronectes americanus*; *Pl* = *Paralichthys lethostigma*; *Sp* = *Symphurus plagiusa*; *Sa* = *Scophthalmus aquosus.*


Growth parameter K
0.35
0.3
0.25
0.2
0.15
0.1
0.05
0
0.2
0.25
0.3
0.35
0.4
0.45
0.5
0.55
0.6
L50/Lmax
Sp
Sa
Pl
Pd2
Pa
Tm
Pd1

mortality based on catch curve data because relatively large numbers of individuals of maximum age were caught.

Symphurus plagiusa is unique among the Cynoglossidae in that it is the only species in the family that inhabits temperate estuarine environments in the seasonally dynamic region of the western north Atlantic. The population in Chesapeake Bay represents the northernmost location of a major population for this species (Munroe 1987). Variability in abiotic conditions increases from southern latitudes to the north, but is highest in the temperate latitudes (Miller et al. 1991; Parr 1933). Abiotic variability also increases towards shore (Miller et al. 1991; Russell-Hunter 1970). Abiotic factors, such as temperature fluctuation, may account for the high level of total instantaneous mortality experienced by this species. Bottom trawls conducted in late winter and early spring of 1996 revealed numerous dead tonguefishes, undoubtedly due to extremely cold water temperatures. Mortality estimates may also be high due to emigration of individuals out of Chesapeake Bay onto the continental shelf. Larger individuals occur on the shelf, but movements of this species have not been studied.

Blackcheek tonguefish exhibit the youngest age at first maturity, the highest growth coefficient, the smallest asymptotic length and the youngest known maximum age when compared to the other pleuronectiforms in this dynamic estuarine system. Blackcheek tonguefish exhibit rapid growth until maturity, after which growth rate declines, and have a high $L_{50}/Lmax$ ratio. Munroe (1987) indicated that eight species of *Symphurus* reach sexual maturity between 28 and 45 mm. Therefore, small size at maturation may be characteristic of fishes of this genus. Blackcheek tonguefish also exhibit highest

TABLE 6

Mortality estimates (Z) for various northwestern Atlantic Pleuronectiformes based on Hoenig's equation, Royce's equation, and catch curve data.

Species	Hoenig's equation	Royce's equation	Catch curve	Data Source
winter flounder (*P. americanus*)	0.32	0.33	0.92	Lux 1973
summer flounder (*P. dentatus*)	0.49	0.51	1.12	Desfosse 1995
windowpane (*S. aquosus*)	0.62	0.66	1.08	Moore 1947
southern flounder (*P. lethostigma*)	0.73	0.77	1.15	Music & Pafford 1984
hogchoker (*T. maculatus*)	0.73	0.77	0.50	Mansuetti & Pauly 1956
blackcheek tonguefish (*S. plagiusa*)	0.87	0.92	1.17	Terwilliger, this study

instantaneous total mortality when compared to the other flatfishes. The other species are relatively K-selected in their growth parameters because they mature at a larger size, have a larger asymptotic length, display lower K values, and have lower L_{50}/L_{max} ratios. Interesting to note, only the small sized and short lived species (*S. plagiusa* and *T. maculatus*) complete their life cycles in Chesapeake Bay. Species with relatively r-selected life history parameters are hypothesized to be better adapted at exploiting seasonally dynamic and highly unpredictable environments. Many of the growth features of small sized flatfishes completing their life cycles within Chesapeake Bay are relatively r-selected, which is consistent with this theory.

CHAPTER 2

Reproductive biology of blackcheek tonguefish, *Symphurus plagiusa*, in Chesapeake Bay, Virginia

INTRODUCTION

The blackcheek tonguefish, *Symphurus plagiusa* (Linnaeus 1776), ranges from Connecticut southward through the Florida Keys, northern Gulf of Mexico, and Bahamas (Ginsburg 1951; Topp and Hoff 1972; Munroe 1987). From Chesapeake Bay southward it is an abundant component of the fish fauna occurring in estuaries and coastal embayments. Juveniles and adults are present in trawl collections from Chesapeake Bay throughout the year, indicating that *S. plagiusa* is a resident species in this system. Although *S. plagiusa* is the only cynoglossid found in Chesapeake Bay (Ginsburg 1951; Musick and Wiley 1972; Munroe 1987) and is the second most abundant pleuronectiform that occurs there (Bonzek et al. 1993), information on the reproductive biology of the species is limited, aside from a few reports detailing aspects of the spawning season (Hildebrand and Cable 1930; Moe and Martin 1965; Topp and Hoff 1972; Olney and Grant 1976; Hettler and Barker 1993). These reports were produced by macroscopic examination of gonads and by seasonal occurrence of larvae and small juveniles in plankton collections. Despite its abundance, little research has been conducted on blackcheek tonguefish; its small size renders it inaccessible to most recreational and commercial fishing gears used in Chesapeake Bay. Length of spawning season, location of spawning, size and age at maturity, and fecundity estimates are unknown for this

species. No detailed descriptions of gonadal maturation stages exist, and detailed histological examination of gonadal tissue has not been performed to supplement macroscopic details.

Several studies have described reproduction and/or maturation for members of the genus *Cynoglossus* from Indian Ocean localities (Seshappa 1974; Seshappa 1978; Ramanathan et al. 1980; Datta and Das 1983; Bal and Rao 1984; Datta and Das 1985; Seshappa and Chakrapani 1988; Rao and Dwivedi 1988; Rao and Dwivedi 1991) and the western Pacific (Fujita et al. 1986; Jiang and Wan 1988). Three studies detailing reproductive biology of *Symphurus* spp. exist, from the Gulf of Mexico (Topp and Hoff, Jr. 1972), Mediterranean Sea (Cau and Deiana 1979), and California (Goldburg 1981). Many of these studies report batch fecundity estimates and provide a gross macroscopic ovarian description, but few have used histological techniques to supplement their data.

Previous attempts to describe ovarian development in *S. plagiusa* during gonadal maturation consisted only of macroscopic examination (Hildebrand and Cable 1930; Moe and Martin 1965; Topp and Hoff 1972). Determination of maturity stages based only on macroscopic examination involves a degree of subjectivity on the part of the examiner. Histological examination of gonadal tissue, in conjunction with macroscopic examination and staging, allows for more exact descriptions of gonadal maturity.

A key issue of fecundity estimation in fishes is whether annual fecundity can be estimated from the standing stock of advanced oocytes in the ovary prior to the onset of the reproductive season (Hunter et al. 1985). In many fish, the total number of eggs to be spawned during a given spawning period is fixed prior to spawning--a condition known as

determinate fecundity. In species that exhibit indeterminate fecundity, annual fecundity cannot be determined by the standing stock of advanced oocytes because unyolked oocytes continue to be matured and spawned throughout a protracted spawning season. Oocytes of fishes that display indeterminate fecundity usually occur in many maturity stages, from small unyolked oocytes to large, hydrated oocytes ready to be spawned. Hildebrand and Cable (1930) noted that eggs removed from ripe or nearly ripe specimens of *S. plagiusa* taken from Chesapeake Bay were of several different sizes, indicating that this species exhibits indeterminate fecundity. A protracted spawning season has also been indicated by collections of blackcheek tonguefish larvae in Chesapeake Bay from late May through early October (Hildebrand and Cable 1930). In other areas, *S. plagiusa* larvae have been collected from May through January in inlets off North Carolina barrier islands (Hettler and Barker 1993), and spawning reportedly occurs from late February through early September for Gulf of Mexico specimens (Topp and Hoff 1972).

Symphurus plagiusa is ideal for life history studies because it is unique among the Cynoglossidae in that it inhabits estuarine environments in the seasonally dynamic region of north temperate latitudes. Although most cynoglossids occur in the tropics, those species that do occur in temperate waters generally are small- sized, deep-water forms that are difficult to catch in abundance.

This paper describes the fecundity, spatial and temporal spawning patterns, size at sexual maturity, sex ratios, and details both macroscopic and microscopic gonadal development for blackcheek tonguefish collected in Chesapeake Bay, Virginia. Understanding the reproductive biology of this species will aid in our understanding of

cynoglossid biology, as well as lend insight into the reproductive biology of other small-sized, estuarine-dependent flatfishes.

MATERIALS AND METHODS

Blackcheek tonguefish were captured via the Virginia Institute of Marine Science (VIMS) juvenile finfish and blue crab trawl survey of the lower Chesapeake Bay and the three major Virginia tributaries: the James, York, and Rappahannock Rivers. Fish were collected from April 1994 through August 1995 using a 9.14 m otter trawl with a 3.81 mm stretch mesh body, 0.64 mm mesh cod end liner, and attached tickler chain. Samples were randomly selected from the catch, brought to the lab, measured for total length (TL) to the nearest millimeter, and total weight to the nearest hundredth of a gram. Ovaries were staged macroscopically and assigned to one of the following stages: immature, resting, developing, ripe, partially spent, and spent. Undifferentiation of male gonadal tissue prevented macroscopic staging of the testes. Macroscopic staging was based on criteria, summarized in Table 7, adopted from Colman (1973).

Both gonads were removed from each fish and weighed to the nearest hundredth of a gram. Testes were placed in Davidson's fixative for histological processing and examination. When ovaries were macroscopically classified as gravid, one randomly-selected ovary was placed in Davidson's fixative for histological preparation, while the other was placed in 10% buffered formalin for fecundity analysis. Ovaries classified as a stage other than gravid were both placed in Davidson's fixative, because fecundity

TABLE 7

Description of macroscopic gonadal staging criteria for female blackcheek tonguefish, *Symphurus plagiusa*, in Chesapeake Bay, Virginia.

Immature:	Ovaries greatly reduced in size and translucent; little posterior elongation. No vascularization apparent and tissue appears undifferentiated.
Developing:	Ovaries increasingly larger in size and posteriorly elongated; with a deep yellow coloration and blackish mottle. Slight vascularization of epithelium. Ovary appear granular due to follicular development.
Gravid:	Ovaries relatively very large, elongate, distended and highly vascularized, appearing granular, with distinct eggs visible through epithelium, which is fragile and easily torn. Coloration a deep yellow.
Partially spent:	Ovaries generally elongated and highly vascularized, with fewer eggs and a slightly deflated appearance. Coloration a darker shade of yellow.
Spent:	Ovaries elongate, but extremely flaccid with few eggs remaining. Coloration a blotchy dark brown, with disrupted vascularization.
Resting:	Ovaries appear light yellow, with some black mottle and evidence of follicular development. Ovaries extended, with no vascularization.

estimates for indeterminate spawners can only be made on hydrated ovaries (Hunter et al. 1985). Ovaries designated for histological examination were left in Davidson's fixative for 24 hr, then placed under running tap water for a minimum of 12 hr. After fixation, a small representative piece from anterior, middle, and posterior regions of the ovary was removed and processed histologically to verify macroscopic staging, assess spawning patterns, and to determine length of spawning season.

Ovary portions and testes preserved in Davidson's fixative were embedded in paraffin, sectioned at 5m and stained using Harris' hematoxylin and counter-stained with eosin Y. All male gonads were examined. Randomly selected ovarian sections representative from each macroscopic maturity stage were examined. Gonad sections were examined to determine stages of spermatogenesis and oogenesis in order to verify accuracy of macroscopic staging and to examine histologically the blackcheek tonguefish gonad. Histological classification of ovarian tissue was based on occurrence and relative abundance of oocytes in the following cellular stages: primary growth, cortical alveoli, partially yolked oocytes, fully yolked oocytes, mature oocytes (which include oocytes undergoing final oocyte maturation and hydrated oocytes), and atretic oocytes .

Oocyte diameters were measured on two gravid females to determine whether blackcheek tonguefish exhibit determinate or indeterminate fecundity. These fecundity patterns were distinguished by plotting a frequency distribution of maximum oocyte diameter. A continuous oocyte size distribution indicates indeterminate fecundity, while a discontinuous size frequency indicates determinate fecundity (Hunter et al. 1985). The ovary was sliced lengthwise and oocytes teased away from the ovarian membrane with

dissecting probes. Oocytes were placed in 10% buffered formalin, stirred to minimize settling bias, and a small sample was pipetted onto a gridded petri dish. Maximum diameters of a random sample of 500 oocytes from each ovary were then measured to the nearest 0.0001 m using the Biosonic Optical Pattern Recognition System.

Microscopic analysis was used to determine length of spawning season by examining relative frequency of gonadal stages in fish caught throughout the year. A gonadosomatic index (GSI), also used to determine overall spawning season, was calculated for each sex using the following equation:

$$GSI = \frac{\text{weight of gonad}}{\text{total weight of fish}} \times 100$$

Chi-square analysis (Zar 1984) was used to determine significant differences from a 1:1 sex ratio for the overall catch data. G-test (Sohkal and Rohlf 1981) was performed to determine significant deviations from 1:1 among 25 mm size groups, month of capture, and location of capture. When number for a sex was zero for any class, Yates' correction for continuity was employed and a constant value of 0.5 was added to all counts for that test.

Mean length at first maturity (L_{50}) was calculated for both sexes by plotting a sigmoid curve to the percent of mature adults by 20 mm length increment using probit analysis in SASS. L_{50} was defined as the smallest length interval in which 50% of the individuals examined were sexually mature. Females were considered sexually mature if the ovary was in a developing or more advanced stage of maturation.

Batch fecundity was determined gravimetrically on twelve females using the hydrated oocyte method (Hunter et al. 1985). All hydrated oocytes from the formalin preserved ovary were counted, and these counts were projected to a total count for both ovaries using the formula:

$$Y = (y/x)\,X$$

where:

Y = fecundity
y = number of hydrated oocytes in the sample
x = weight of the tissue sample
X = ovarian total weight

The small size of preserved ovaries allowed for a count of all hydrated oocytes in that ovary; no subsampling was needed. Counting all hydrated oocytes in a randomly selected ovary also precluded the need for statistical analyses to determine positional effects within and between ovaries. Simple linear regressions were used to describe relationships between batch fecundity and total length (TL) and between batch fecundity and total weight (TW). Single factor analysis of variance (ANOVA) was employed to test if larger females have greater relative fecundity and produce more eggs per gram total weight than do smaller females.

Spawning frequency, the number of times a female spawns during the spawning period, was calculated by dividing the total number of females ready to spawn during the spawning period by the total number of females caught during that period. Total annual fecundity of females was then calculated by the formula:

$$T = b \times s$$

where T = Total annual fecundity

b = batch fecundity

s = # spawns during the spawning period

A 24-hr survey was attempted in July 1995 to determine daily spawning periodicity. Complete sampling through a 24-hr period was not possible due to poor weather conditions. Specimens were collected over a 12-hr period from 1020 to 2220. Five minute bottom trawls occurred once hourly, unless the catch failed to yield a mature female tonguefish; in those instances, trawls were repeated until a female was collected. After an incision was made along the length of the ovary, collected females were placed in 10% buffered formalin. After sampling, specimens were stepped up in progressively stronger alcohol concentrations and left in 70% ETOH for preservation. Ovaries were then removed and prepared for histological examination to determine daily spawning periodicity.

RESULTS

External sexual dimorphism was not apparent in *S. plagiusa*. There were no differences in size, shape, or coloration between sexes. Under certain conditions, sex of the individual could be determined by examining the blind side of the fish. In small, thin fish the ovary could be seen when the fish was held up to light, and when the fish was a developing or gravid female, the ovary was so distended as to be visible as a long bulge along the ventral length of the fish.

The gonads of male and female blackcheek tonguefish are dissimilar, and sex is easily determined by macroscopic examination. Maturing females display long, tubular ovaries that extend posteriorly into a secondary body cavity (Ochiai 1963), especially as the fish matures (Fig. 12). Immature females display transparent, short ovaries that barely extend into the secondary body cavity. Ovaries connect anteriorly, immediately posterior to the body cavity, and are physically separated throughout most of their length by musculature and the haemal spines of the vertebral column. Size and coloration of the ovaries depends heavily upon seasonal maturity stage of the female (Table 7). Males have small, white, pea-shaped testes suspended by mesenteric connective tissue in the posterior portion of the body cavity (Fig. 13). Testes are physically separated by the anteriormost haemal spine, and are positioned such that the sperm ducts face the posterior wall of the body cavity and curve to meet the urogenital pore. Lack of differentiation into distinct maturity

Figure 12. Macroscopic appearance of developing ovaries of blackcheek tonguefish, *Symphurus plagiusa*, from Chesapeake Bay.

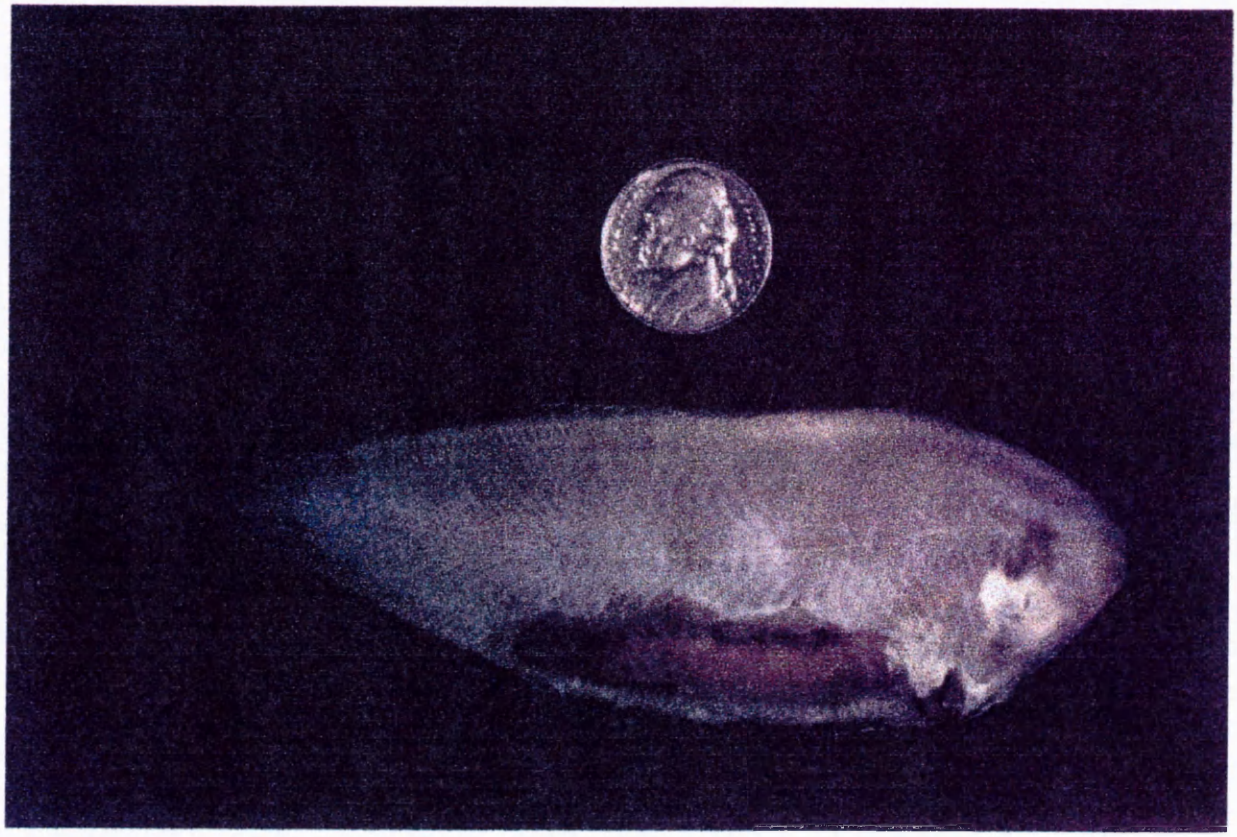
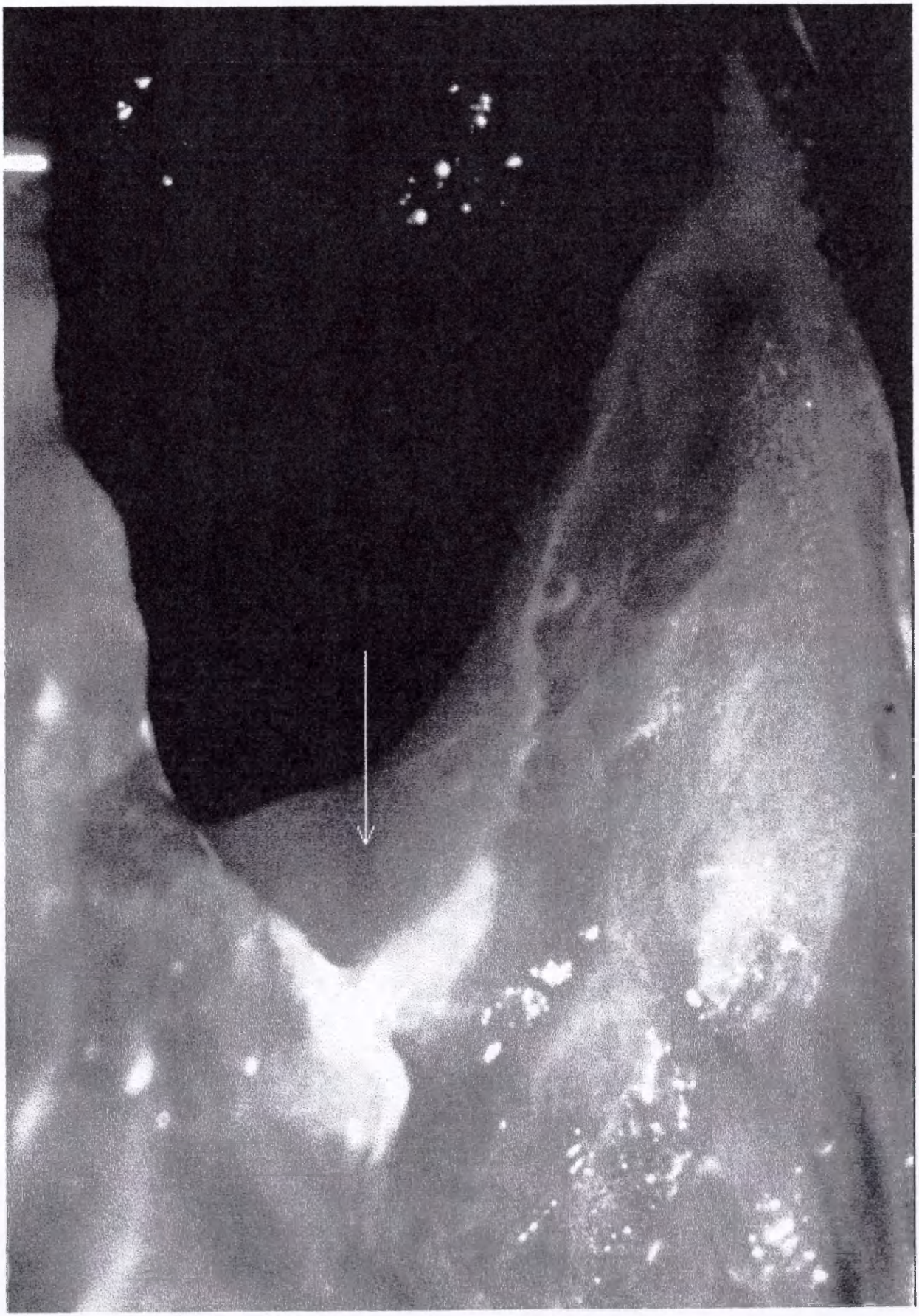


Figure 13. Macroscopic appearance of testes of blackcheek tonguefish, *Symphurus plagiusa*, from Chesapeake Bay.



stages precluded macroscopic staging of testes. Macroscopically, it was impossible to distinguish between a mature and an immature male.

The principal cytologic changes observable during oogenesis permit identification of seven stages of development: immature, developing, fully developed, gravid, partially spent, spent, and resting. The immature ovary (Fig. 14) is characterized by a thin ovarian capsule, the presence of only primary growth oocytes, and no atresia. Primary growth oocytes are extremely basophilic and are characterized by their centrally located nucleus and scant cytoplasm. The developing ovary (Fig. 15) exhibits primary growth, cortical alveoli, the possibility of a few partially yolked oocytes, and no major atresia. Cortical alveoli are clear vesicles of various size which contain protein and PAS positive substances in fluid released at the time of fertilization (Wallace and Selman 1981). Partially yolked oocytes are those that are in the early stage of vitellogenesis. In these oocytes, acidophilic yolk bodies begin to accrue in the oocyte. The fully developed ovary (Fig. 16) consists of primary growth, cortical alveoli, and partially and fully yolked oocytes. The central part of the fully yolked oocyte is occupied by a mass of fluid yolk. Atresia of fully yolked oocytes may be present. Atretic oocytes are those that are being resorbed by the follicle; they are characterized by a disorganized cellular structure and shrinkage of the zona radiata away from the theca. The gravid ovary (Fig 17) is characterized by the presence of the previously mentioned cell types as well as hydrated oocytes. Hydrated oocytes are easy to distinguish histologically because of their wrinkled appearance. Oocytes undergoing final oocyte maturation (FOM) (Fig. 18) were also included in this stage because this stage occurs just previous to the oocytes becoming hydrated. FOM oocytes

Figure 14. Histological appearance of immature stage blackcheek tonguefish ovary: PG = primary growth oocyte.

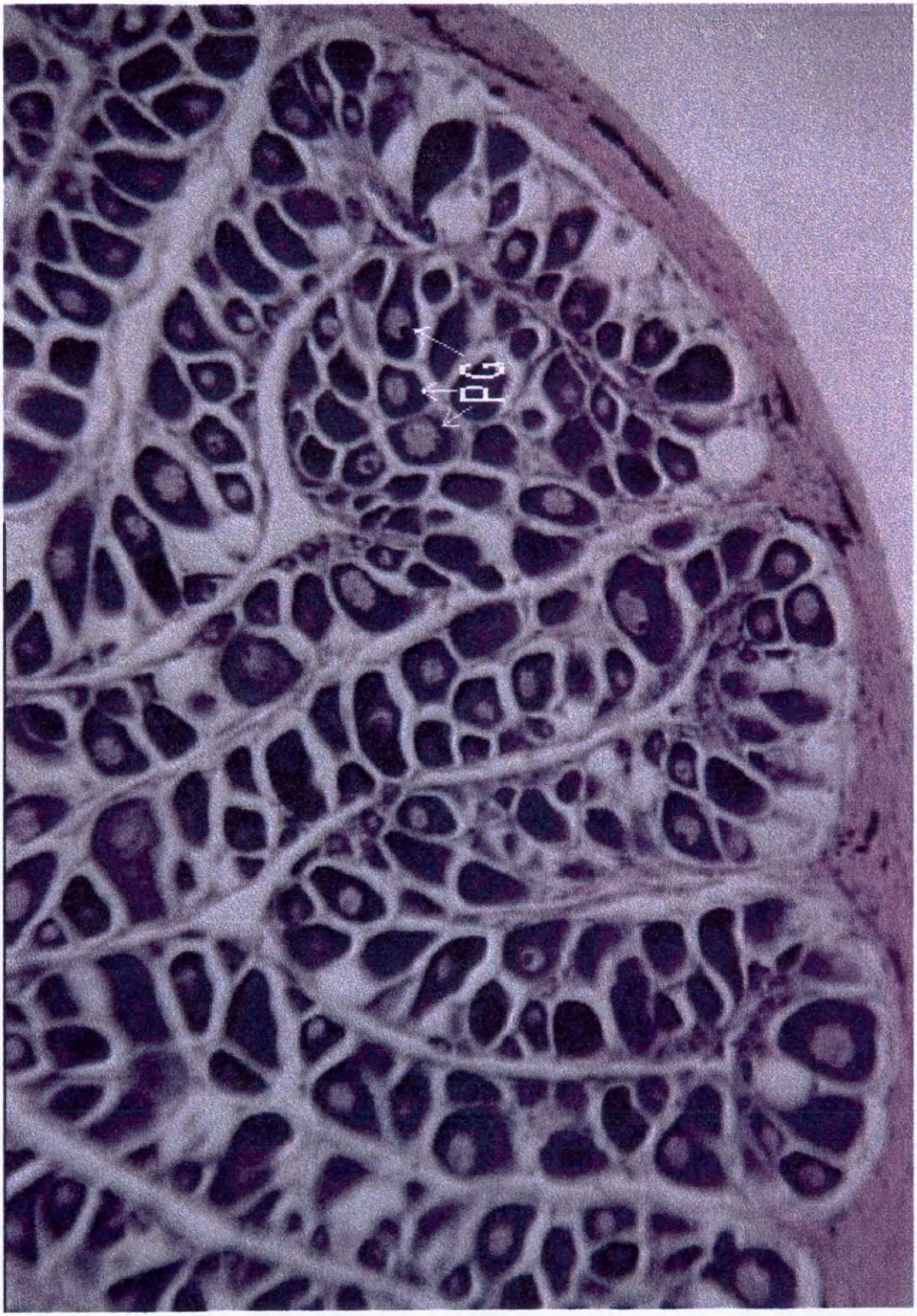


Figure 15. Histological appearance of developing stage blackcheek tonguefish ovary: PG = primary growth oocyte; CA = cortical alveoli.

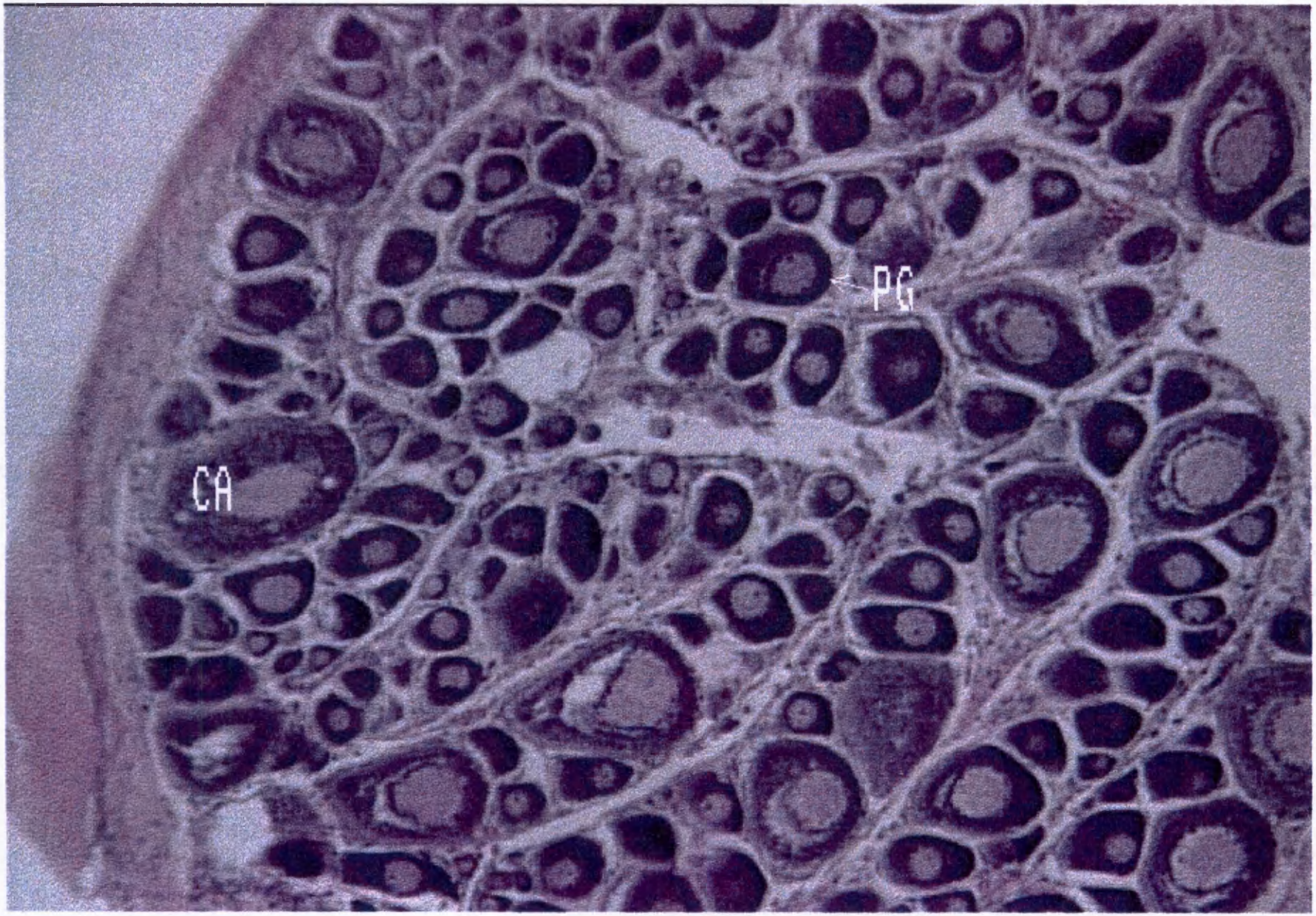

PG
CA

Figure 16: Histological appearance of fully developed stage blackcheek tonguefish ovary: CA = cortical alveoli; PY= partially yolked oocyte; FY = fully yolked oocyte.

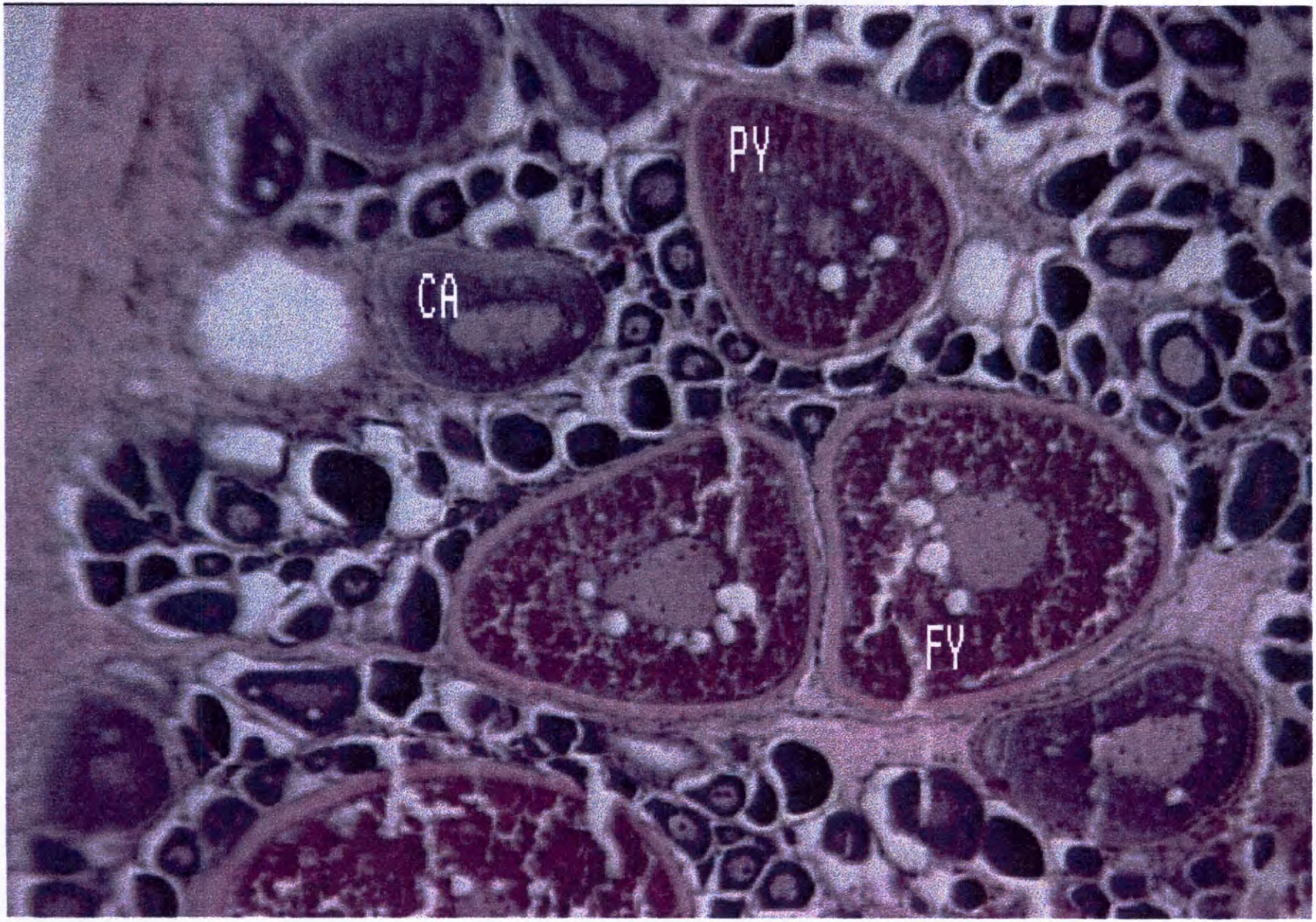

PY
CA
FY

Figure 17. Histological appearance of gravid stage (hydrated) blackcheek tonguefish ovary: FY = fully yolked oocyte; HO = hydrated oocyte.

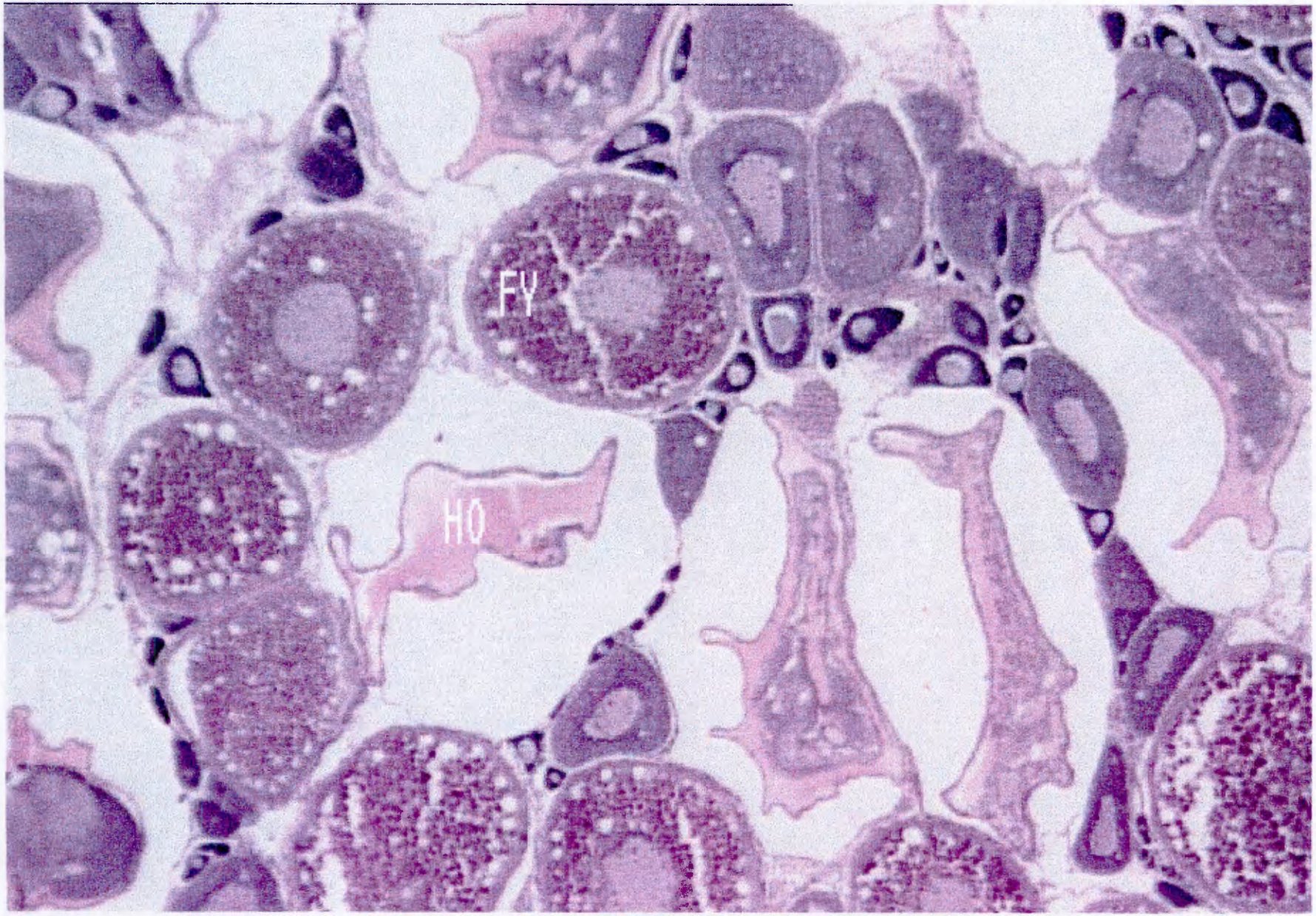
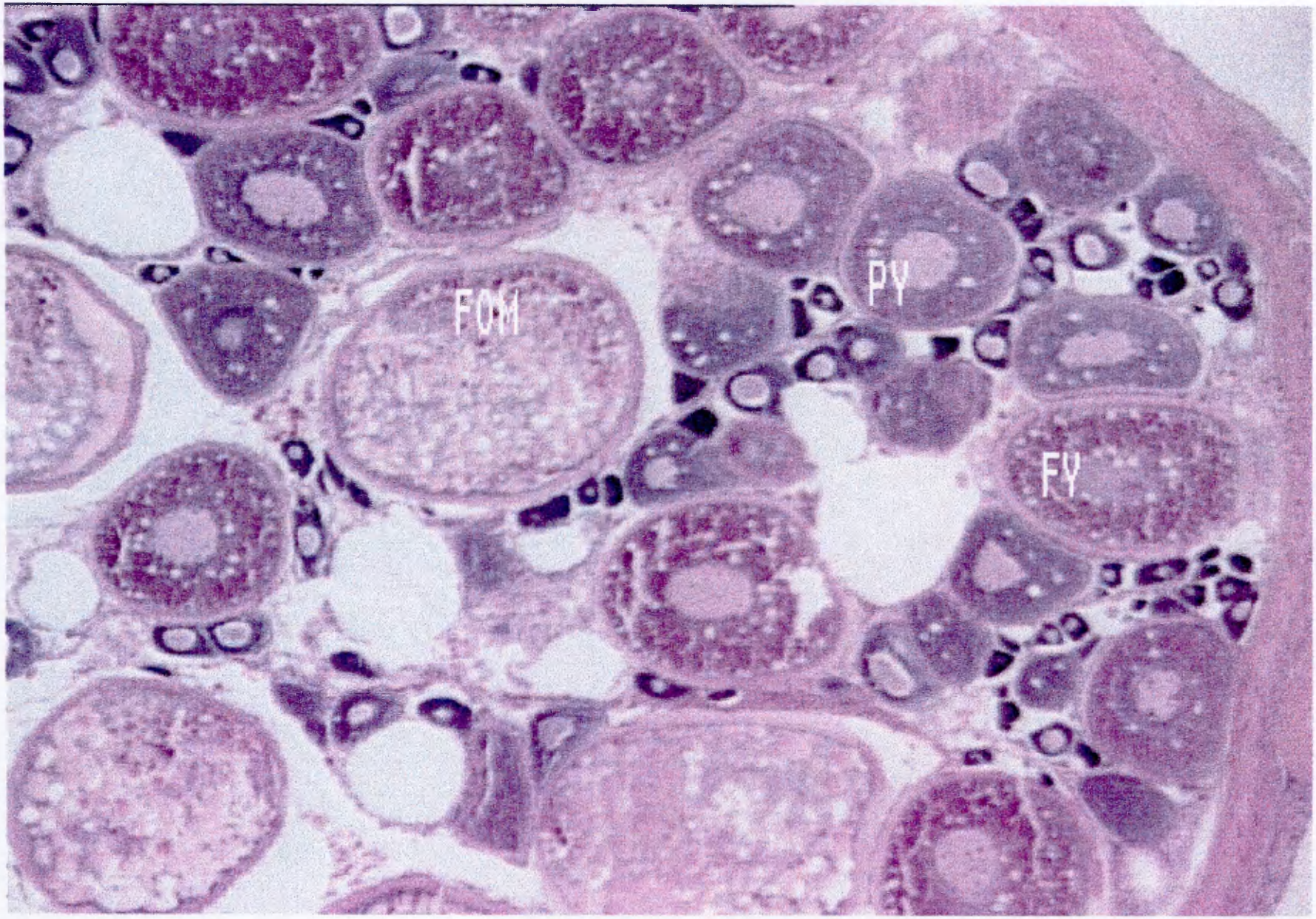

FY
HO

Figure 18. Histological appearance of gravid stage (final oocyte maturation) blackcheek tonguefish ovary: PY = partially yolked oocyte; FY = fully yolked oocyte; FOM = final oocyte maturation.


FOM
PY
FY

are large, amorphous oocytes in which the yolk globules have begun to undergo hydration and fusing lipid vacuoles appear as large, clear areas. The partially spent ovary (Fig. 19) is characterized by primary growth through fully yolked oocytes, with atresia occurring throughout the ovary. The presence of post-ovulatory follicles is the usual criterion for classifying ovaries as partially spent; however, no post-ovulatory follicles were found in histological sections, suggesting that these structures are short-lived and are resorbed soon after oocytes are spawned. The spent ovary (Fig. 20) exhibits major atresia throughout the ovary. The resting ovary (Fig. 21) is characterized by the presence of a thick capsule composed of connective tissue (tunica albuginea) and only primary growth oocytes. No fish in 'running-ripe' condition were found.

The male reproductive cycle is quite different than that of the female, and is characterized by four main cell types: spermatogonia, spermatocytes, spermatids, and spermatozoa. The immature testis contains only primary spermatogonia. Primary spermatogonia are pale-staining cells with a large central nucleus that contains one or two distinct nucleoli (Selman and Wallace 1986). As they mature, testes develop secondary spermatogonia. Secondary spermatogonia can be distinguished from primary spermatogonia by their smaller size and greater number. Secondary spermatogonia develop into primary spermatocytes, which then develop into secondary spermatocytes. Secondary spermatocytes divide to produce highly basophilic spermatids, which then develop a long flagellum and differentiate into spermatozoa. Spermatozoa can be differentiated from spermatids by their bullet-shaped heads and acidophilic tails.

Figure 19. Histological appearance of partially spent stage blackcheek tonguefish ovary: PY = partially yolked oocyte; FY = fully yolked oocyte; AO = atretic oocyte.

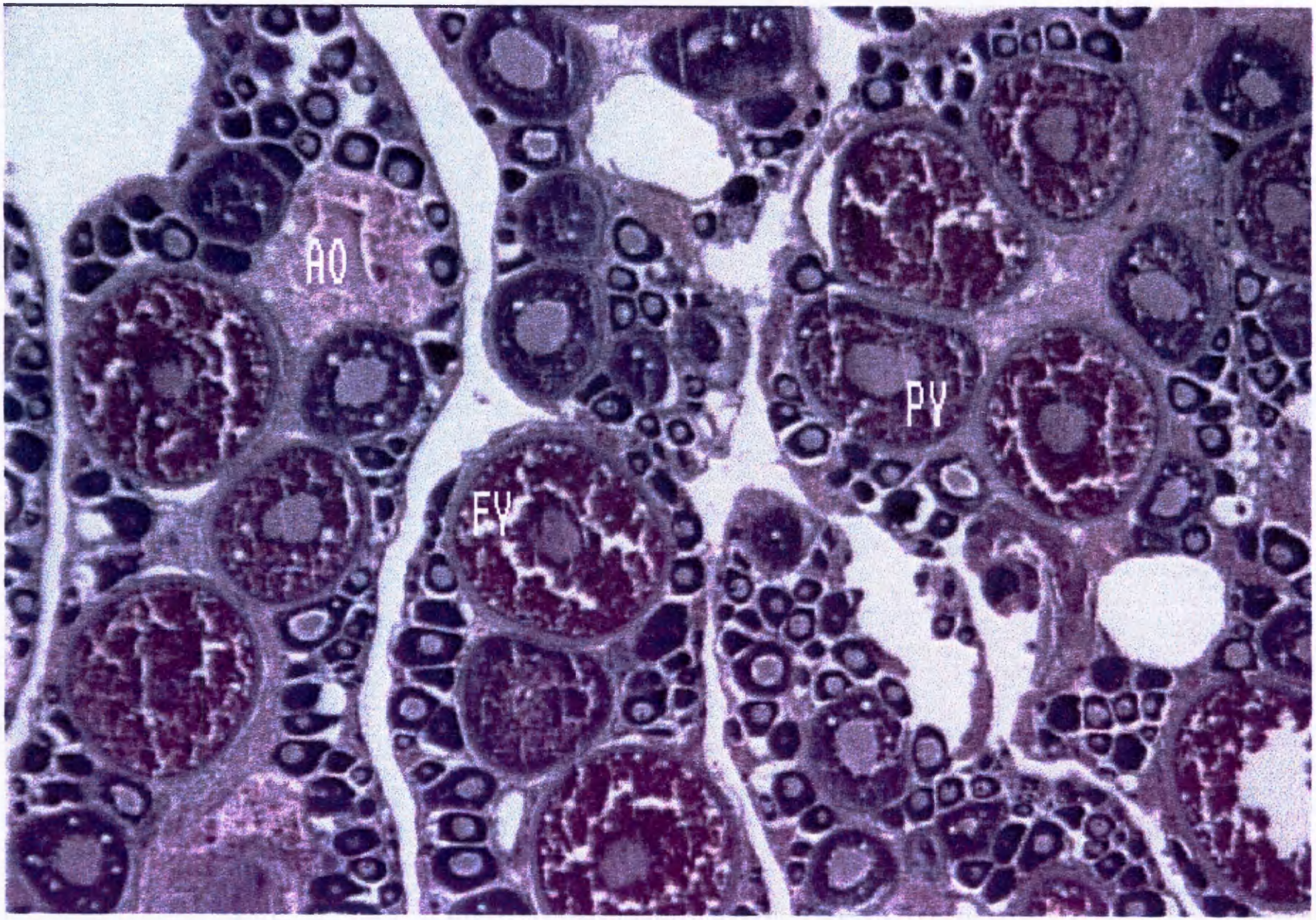

AO
FV
PV

Figure 20. Histological appearance of spent stage blackcheek tonguefish ovary:

AO = atretic oocyte.

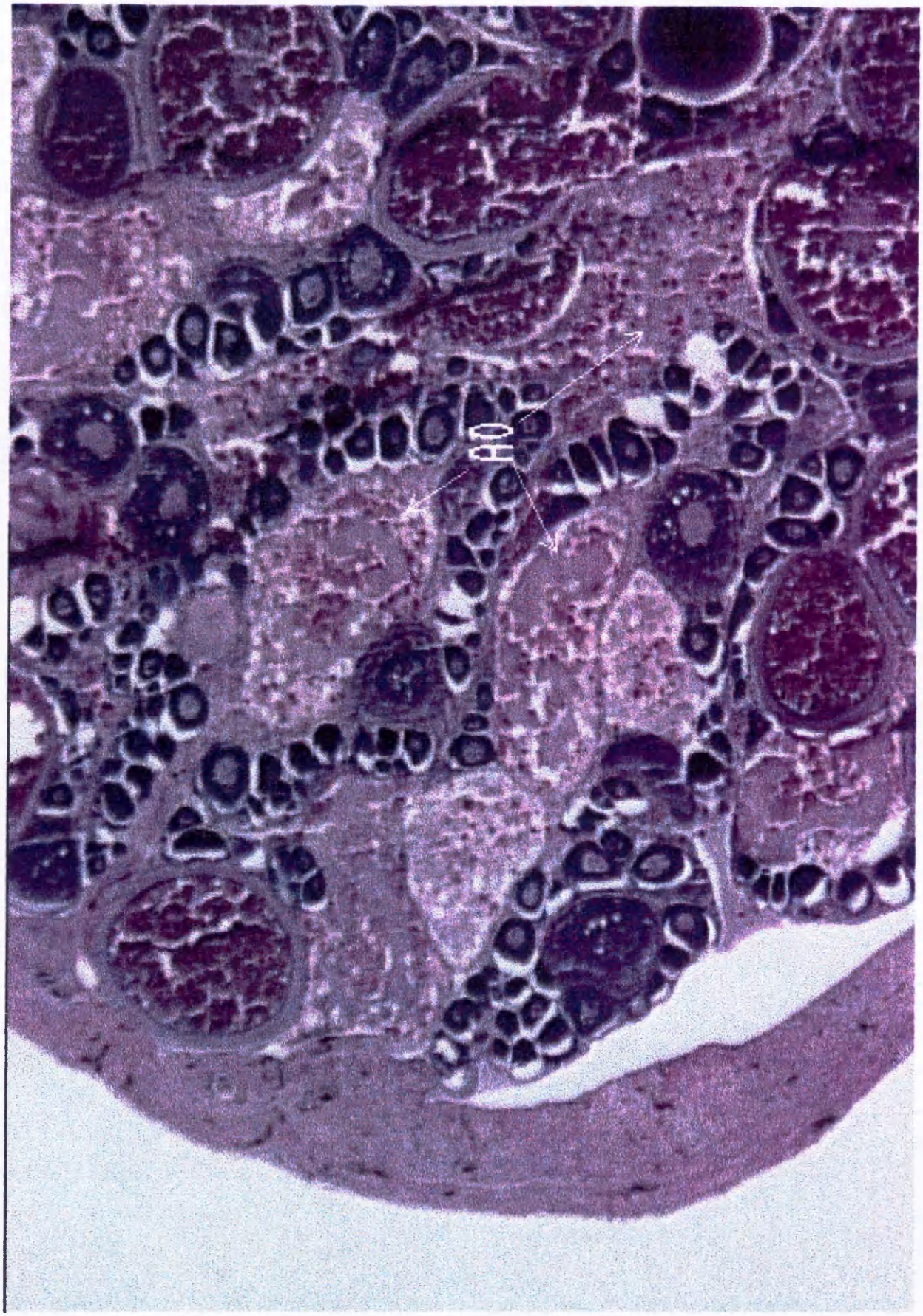
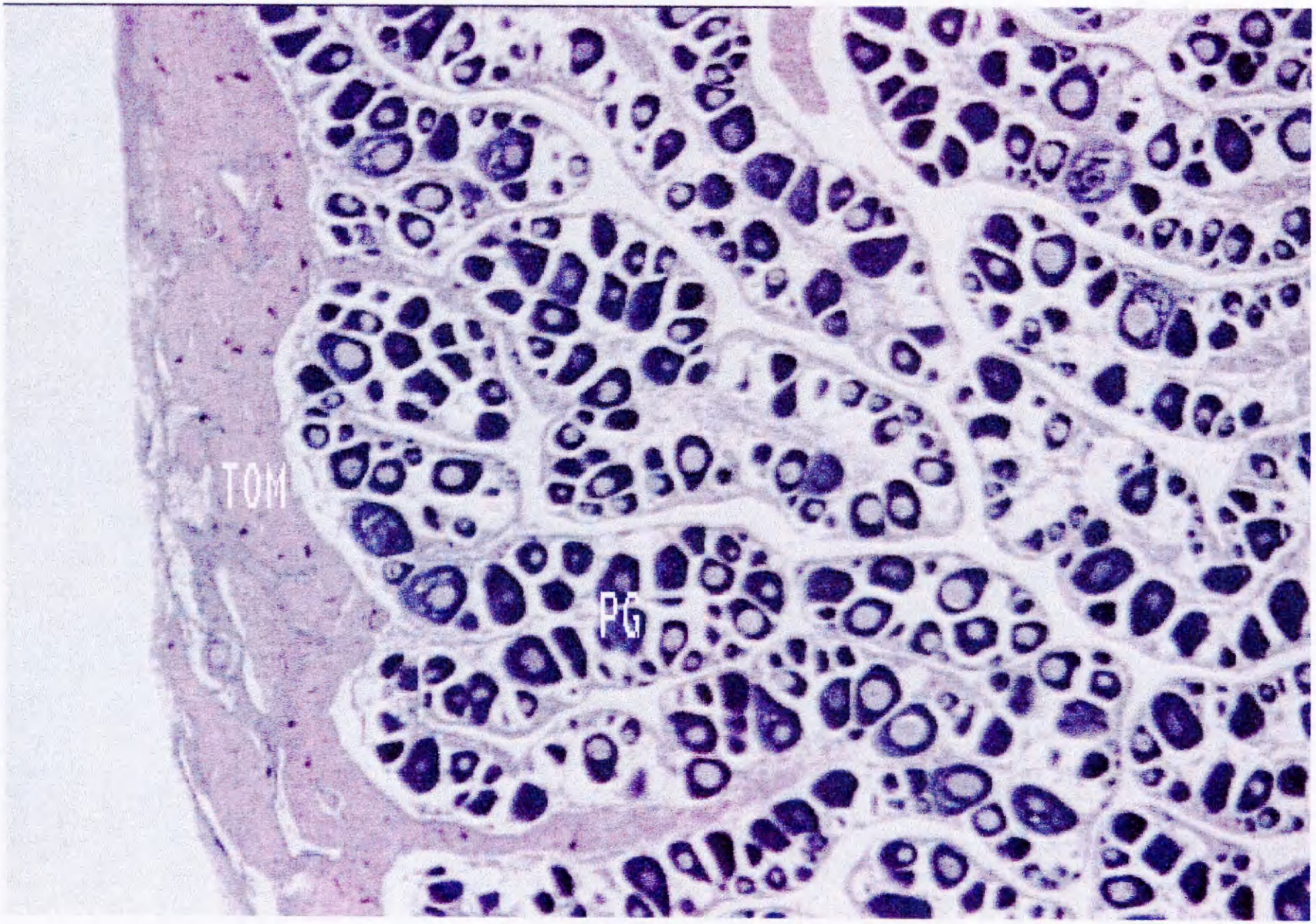

AO

Figure 21. Histological appearance of resting blackcheek tonguefish ovary: PG = primary growth oocyte; TOM = tunica albuginea.


TOM
PG

In an attempt to classify testes based upon the distribution of spermatogonia, Grier (1981) described two main types of testis structure. In the restricted spermatogonial testis, typical in the Atheriniform fishes, spermatogonia are restricted to the distal terminus of the testis lobule, immediately beneath the tunica albuginea. In the unrestricted spermatogonial testis, typical of most teleosts, spermatogonia may occur along the entire length of the lobule. The testes of *S. plagiusa* are lobular, and primary spermatogonia appear to be restricted to the distal ends of the lobule. This type testis therefore resembles the restricted spermatogonial testis (Grier 1981). However, no definite progression of development was seen in histological sections. Therefore, the testis of S. plagiusa cannot be easily accommodated in the classification of Grier (1981) and may represent a morphologically distinct type. Large clusters of cells thought to be Leydig cells are also found throughout the testes (Grier, pers. comm.). Sperm ducts, supported by mesenteric tissue, are found along the periphery of the testes. Sperm ducts of mature males contain dense quantities of spermatozoa, and lesser amounts of spermatogonia, spermatocytes, and spermatids. This atypical teleost gonad structure made histological staging difficult, so males were classified as either immature (Fig. 22) or mature (Fig. 23) based upon the presence of spermatozoa in the testes.

Sampling began in April 1994, when 65% of the females were undergoing ovarian development (Fig. 24). Gonadal recrudescence was observed in a small percentage of females captured in March 1995 and by May, 55% of the females were undergoing ovarian development. Fish with gravid ovaries were observed during June-September 1994, and again during June-August 1995. Partially spent fish (females that appeared to

Figure 22. Histological appearance of immature blackcheek tonguefish testis: PG = primary spermatogonia; PS = spermatocytes.

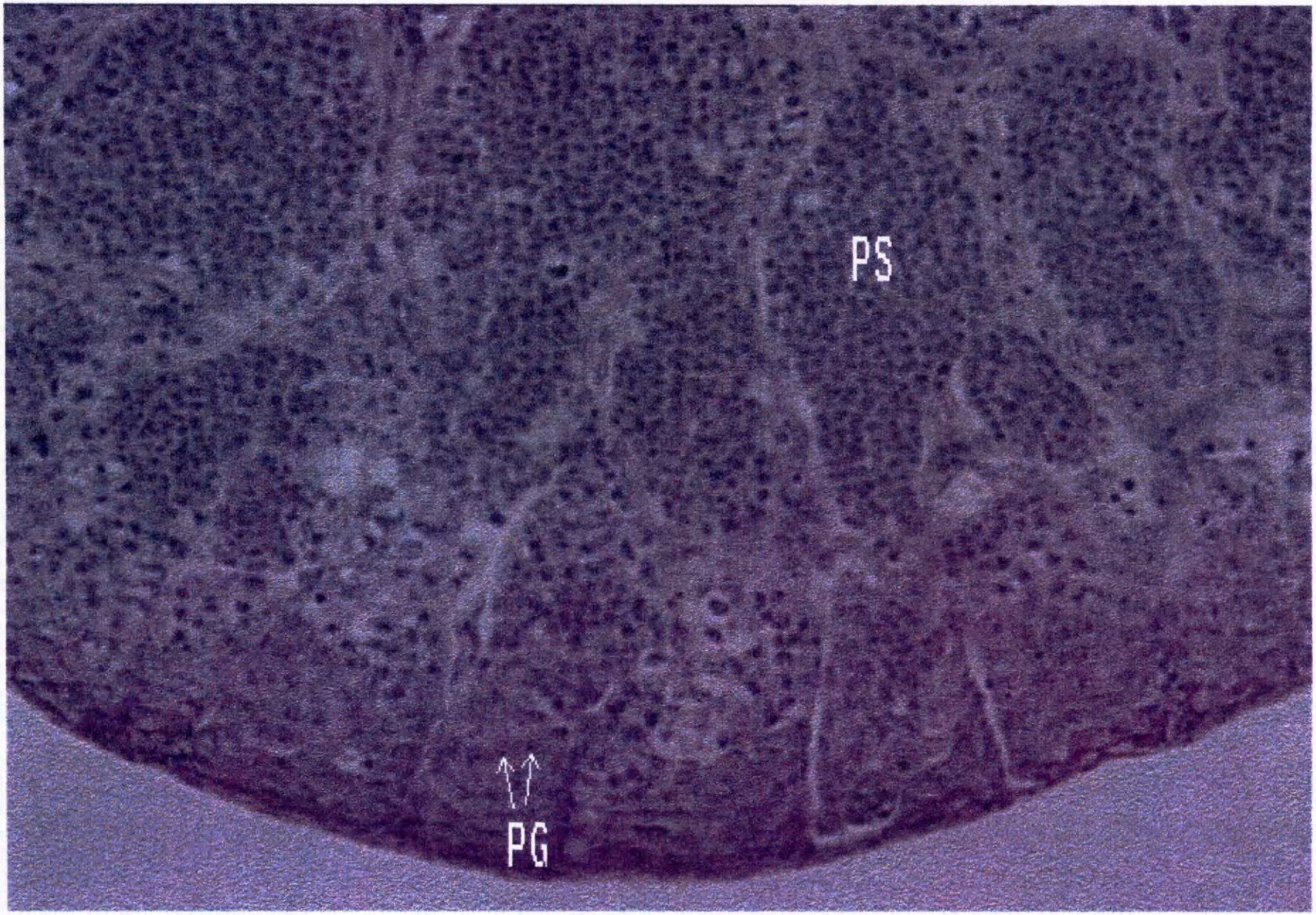

PS
PG

Figure 23. Histological appearance of mature classification blackcheek tonguefish testis: SG = spermatogonia; SC = spermatocytes; ST = spermatids; SZ = spermatozoa; LC = possible Leydig cells.

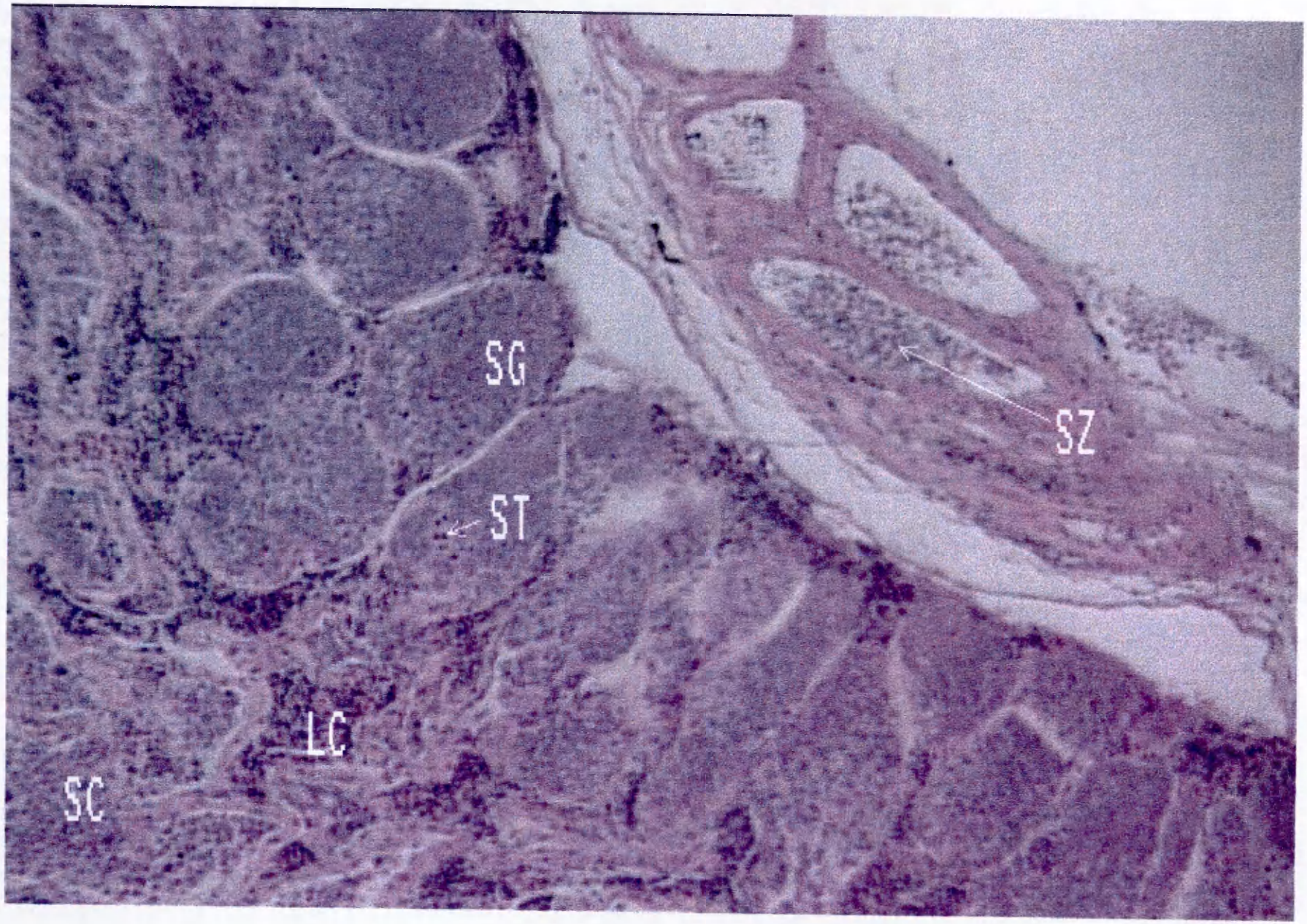
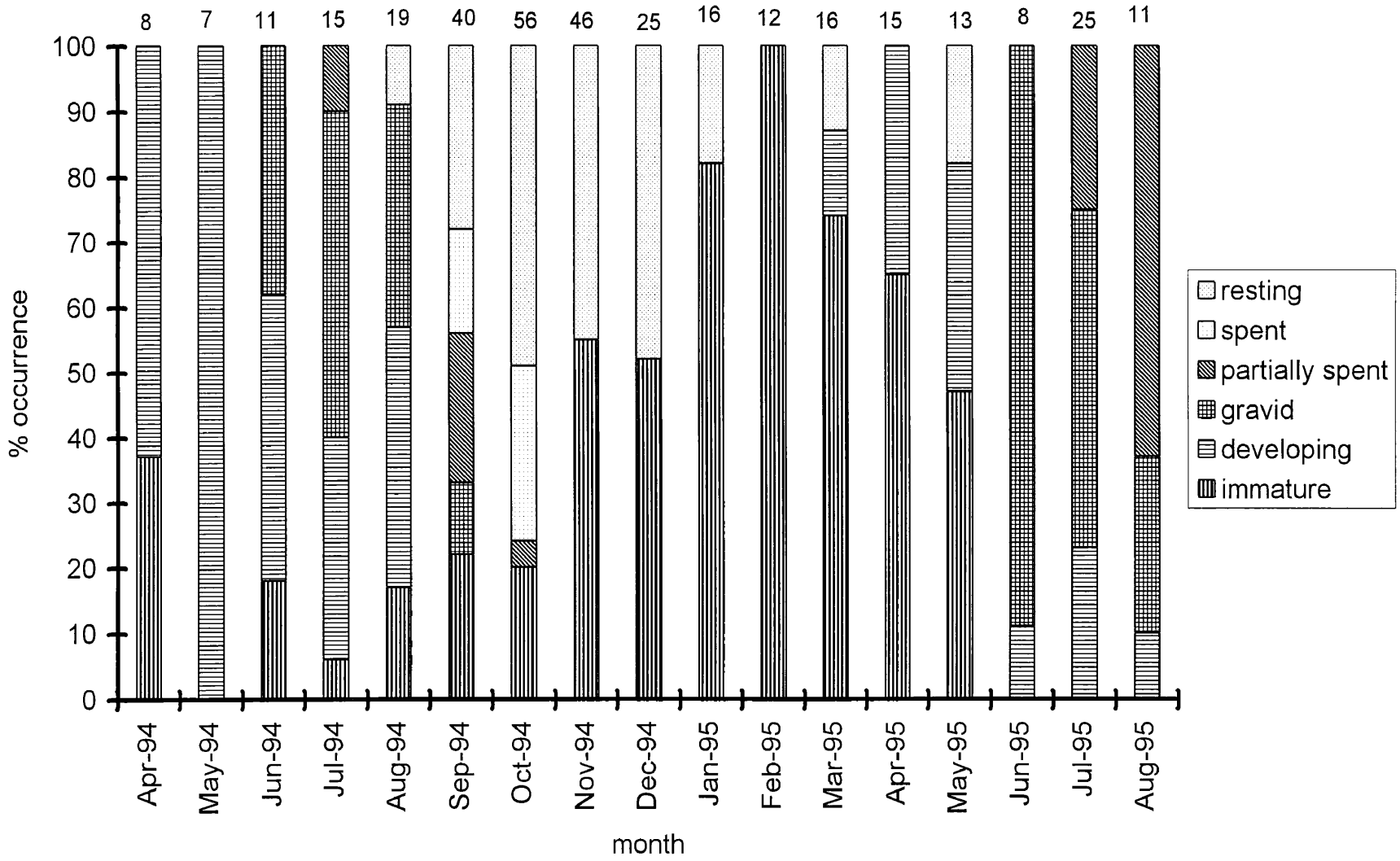

SG
SZ
ST
LG
SC

Figure 24. Frequency of macroscopic gonad stages for female blackcheek tonguefish, *Symphurus plagiusa*, in Chesapeake Bay by month, 1994-95. Numbers at top of figure represent numbers staged each month.


8
7
11
15
19
40
56
46
25
16
12
16
15
13
8
25
11
100
90
80
70
60
50
40
30
20
10
0
% occurrence
Apr-94
May-94
Jun-94
Jul-94
Aug-94
Sep-94
Oct-94
Nov-94
Dec-94
Jan-95
Feb-95
Mar-95
Apr-95
May-95
Jun-95
Jul-95
Aug-95
month
resting
spent
partially spent
gravid
developing
immature

have spawned at least once but still contained vitellogenic oocytes) were captured during July-October 1994 and July-August 1995. No completely spent fish were collected before September or after October 1994. Resting females were collected from August 1994 through May 1995. Mature males were collected throughout the year, with no distinct seasonal differences in gonad stage. Only 8 of 121 males (7%) randomly selected for histological analysis were immature.

Overall, there was 77% agreement between macroscopic and microscopic staging of female reproductive tissue (Fig. 25). Agreement was highest in the immature and partially spent stages (100% agreement), because immature ovaries are relatively small, thin, and translucent, and partially spent ovaries have a noticeably deflated appearance with visible eggs. Agreement was also relatively high with ovaries in the developing stage (71%) and gravid stage (70%) because developing and gravid ovaries are relatively easy to discern. Developing ovaries are bright yellow and vascularized but have no visible eggs, while gravid ovaries are relatively large and distended and eggs are visible through the epithelium. Percent agreement between macroscopic and microscopic staging was relatively low with regard to spent ovaries (36%) and relatively high with resting ovaries (85%). Many discrepancies between stages occurred when ovaries were macroscopically classified as gravid, but were microscopically determined to be developing, and when ovaries macroscopically classified as spent were actually resting (Table 8).

Sex ratios for blackcheek tonguefish collected in this study were 223 males:343 females, or 1:1.54 (Table 1). Chi-square analysis of sex ratios indicated statistically significant deviations from a 1:1 sex ratio. Females dominated many intermediate size

Figure 25. Percent agreement between macroscopic and microscopic ovary stages in blackcheek tonguefish, *Symphurus plagiusa*, from Chesapeake Bay. Numbers at top of figure represent numbers staged.

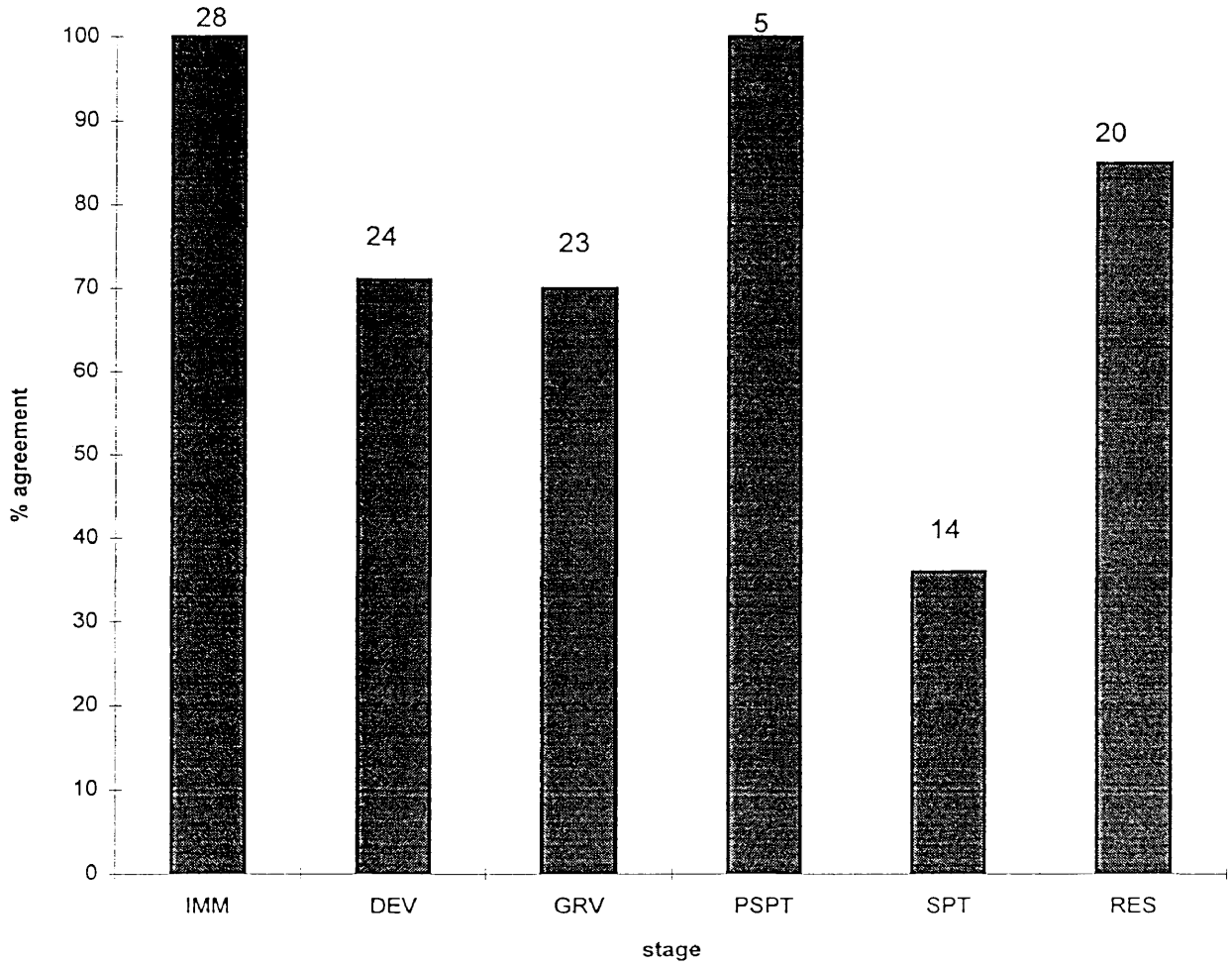

28
5
20
24
23
14
100
90
80
70
60
50
40
30
20
10
0
% agreement
IMM
DEV
GRV
PSPT
SPT
RES
stage

TABLE 8

Summary of representative subsample of microscopic stages assigned to macroscopic ovary stages of blackcheek tonguefish, *Symphurus plagiusa*, in Chesapeake Bay. Data expressed as numbers of ovaries per stage. IM = immature, DE = developing, GRV = gravid, PSPT = partially spent, SPT = spent, RE = resting.

	macroscopic stage					
microscopic stage	IM	DE	GRV	PSPT	SPT	RE
IM	28	1				3
DE		17	6			
GRV			16			
PSPT		3		5	1	
SPT		1	1		5	
RE		2			8	17

classes, summer catches, and catches in the York River and lower Chesapeake Bay. G-tests of sex ratios divided into 25 mm size classes (Table 2) show significant deviations from a 1:1 ratio in the 51-75 mm and 101-175 mm size classes. Females dominated these size classes. Because significant deviations occur for one-half of the size classes, the total G is highly significant. The pooled G, in view of the consistent trend in favor of females, is also highly significant. The heterogeneity G, being non-significant, shows that deviations from expectation were in the same direction and not significantly different from each other. G-tests of sex ratios broken down by month of capture (Table 9) show significant deviations from a 1:1 sex ratio in December 1994, and in May-July 1995. Large numbers of females were captured during these months. Accordingly, the values of the total, pooled, and heterogeneity G are highly significant. The trend in sex ratios consistently favors females, and this trend, although in most cases favoring females, is not uniform in magnitude. G-tests of sex ratios broken down by location of capture (Table 10) show significant deviations from a 1:1 sex ratio for the York River and lower Chesapeake Bay, where females were collected much more often than males. These significant deviations explain the highly significant total G. The pooled G for location effects is also highly significant. The sex ratio trend is consistently in favor of females. The non-significant heterogeneity G shows that the deviations from expectation were in the same direction and not significantly different from each other.

Blackcheek tonguefish in Chesapeake Bay reach sexual maturity at a small size and an early age (Fig. 3). Female blackcheek tonguefish were observed to mature at 80 mm and all were mature by 130 mm. Length at which 50% of the population was mature (L_{50}) was

TABLE 9

G-test of sex ratios for blackcheek tonguefish, *Symphurus plagiusa*, from Chesapeake Bay: analyzed by date of capture

date	males	females	n	df	G	P
Apr-94	7.5	8.5	15	1	0.062541	ns
f(x)	8	8				
May-94	5.5	7.5	12	1	0.308918	ns
f(x)	6.5	6.5				
Jun-94	10.5	11.5	21	1	0.04547	ns
f(x)	11	11				
Jul-94	21.5	15.5	36	1	0.977283	ns
f(x)	18.5	18.5				
Aug-94	10.5	19.5	29	1	2.742032	ns
f(x)	15	15				
Sep-94	27.5	40.5	67	1	2.500659	ns
f(x)	34	34				
Oct-94	45.5	56.5	101	1	1.188585	ns
f(x)	51	51				
Nov-94	43.5	63.5	106	1	3.760396	ns
f(x)	53.5	53.5				
Dec-94	4.5	25.5	29	1	16.22628	P0.001=10.828
f(x)	15	15				
Jan-95	17.5	15.5	32	1	0.121286	ns
f(x)	16.5	16.5				
Feb-95	11.5	12.5	23	1	0.041679	ns
f(x)	12	12				
Mar-95	9.5	16.5	25	1	1.90807	ns
f(x)	13	13				
Apr-95	8.5	15.5	23	1	2.071646	ns
f(x)	12	12				
May-95	4.5	13.5	17	1	4.709233	P0.05=3.841
f(x)	9	9				
Jun-95	0.5	8.5	8	1	8.614584	P0.005=7.879
f(x)	4.5	4.5				
Jul-95	2.5	20.5	22	1	16.07091	P0.001=10.828
f(x)	11.5	11.5				
total	231	351	582	total 16	61.34958	P0.001=39.252
f(x)	291	291		pooled 1	24.92318	P0.001-10.828
				heterogeneity 15	36.4264	P0.005=32.801

TABLE 10

G-test of sex ratios for blackcheek tonguefish, *Symphurus plagiusa*, from Chesapeake Bay: analyzed by area of capture

system	males	females	n	df	G	P
James	16	25	41	1	1.99179	ns
f(x)	20.5	20.5				
York	67	119	186	1	14.73319	P0.001=10.828
f(x)	93	93				
Rapp	4	11	15	1	3.39696	ns
f(x)	7.5	7.5				
CB	134	190	324	1	9.727788	P0.005=7.879
f(x)	162	162				
total	223	343	566	total 4	29.84973	P0.001=18.467
f(x)	283	283		pooled 1	25.63698	P0.001=10.828
				heterogeneity 3	4.212748	ns

101 mm. Males were observed to mature at 70 mm and were all mature by 110 mm. The L_{50} for males was 91 mm. The L_{50} measurements correspond to a large age-0 or small age-1 fish.

Spawning of blackcheek tonguefish occurred in the lower portion of Chesapeake Bay. Females with hydrated oocytes were collected in the deeper (>12 m), more saline areas (>25 ppt) of lower Chesapeake Bay near the Bay mouth, while mature males were caught throughout the study area. Although gravid females were collected throughout the spawning season, they occurred in relatively low numbers; only 32 gravid females were collected, and all of these fish were captured proximal to the mouth of Chesapeake Bay. No females in the gravid stage of ovarian development were found in less saline river systems or in the eastern portion of Chesapeake Bay.

Histological and macroscopic examination of the gonads and mean GSI values indicate that blackcheek tonguefish have an extended reproductive season in Chesapeake Bay, from June through September, with some individuals also spawning into early October. Females captured prior to June had ovaries in immature, resting, or developing stages. Gravid and partially spent ovaries were found only in those females caught during the spawning period. Females collected after the spawning period had ovaries in the spent, resting, and immature stages. Gonadosomatic indices, calculated for 202 males (Fig. 26) and 308 females (Fig. 27), also show that blackcheek tonguefish spawn from June through October, with increases in GSI occurring as early as May. Female GSI values peaked in July 1994 (1.38) and June 1995 (1.75). Male GSI values peaked in July 1994 (.037) and June-July 1995 (.069), and decreased steadily to a low in January (0.005). The absence of

Figure 26. Monthly mean gonadosomatic indices and range for male blackcheek tonguefish, *Symphurus plagiusa*, from Chesapeake Bay.

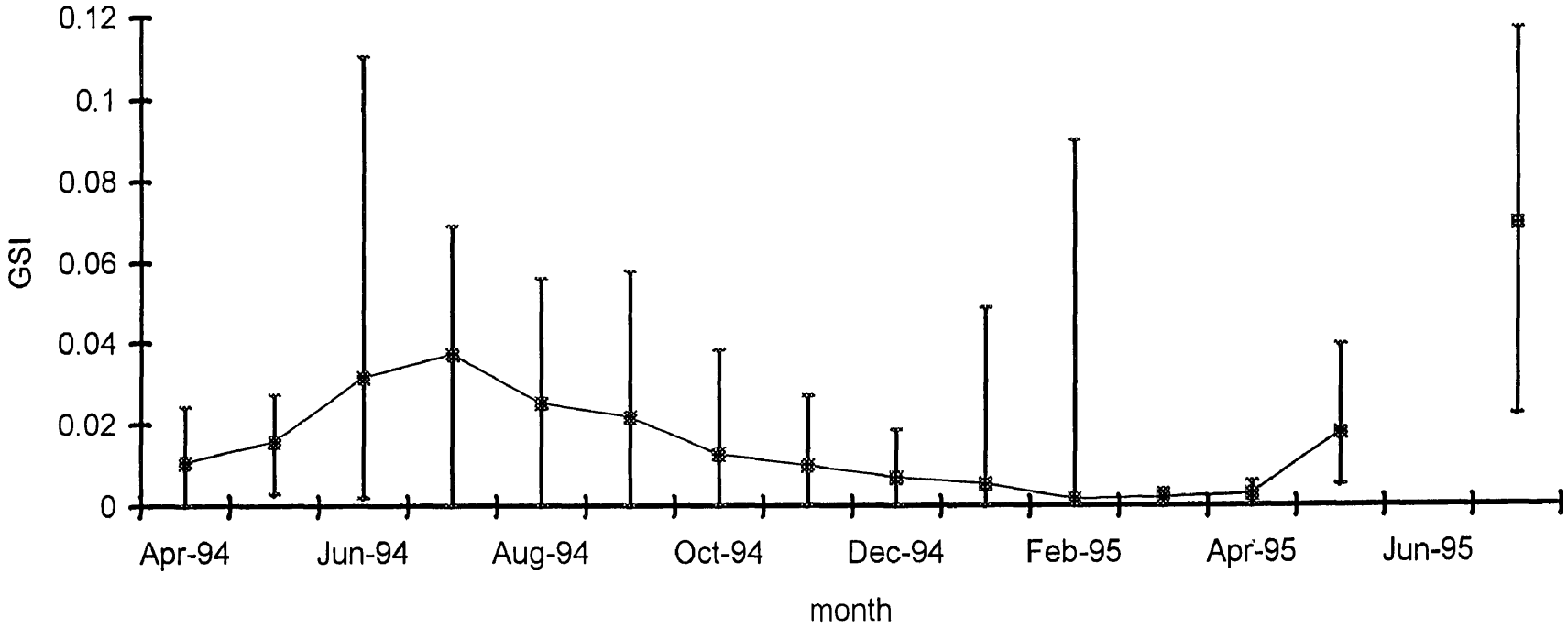
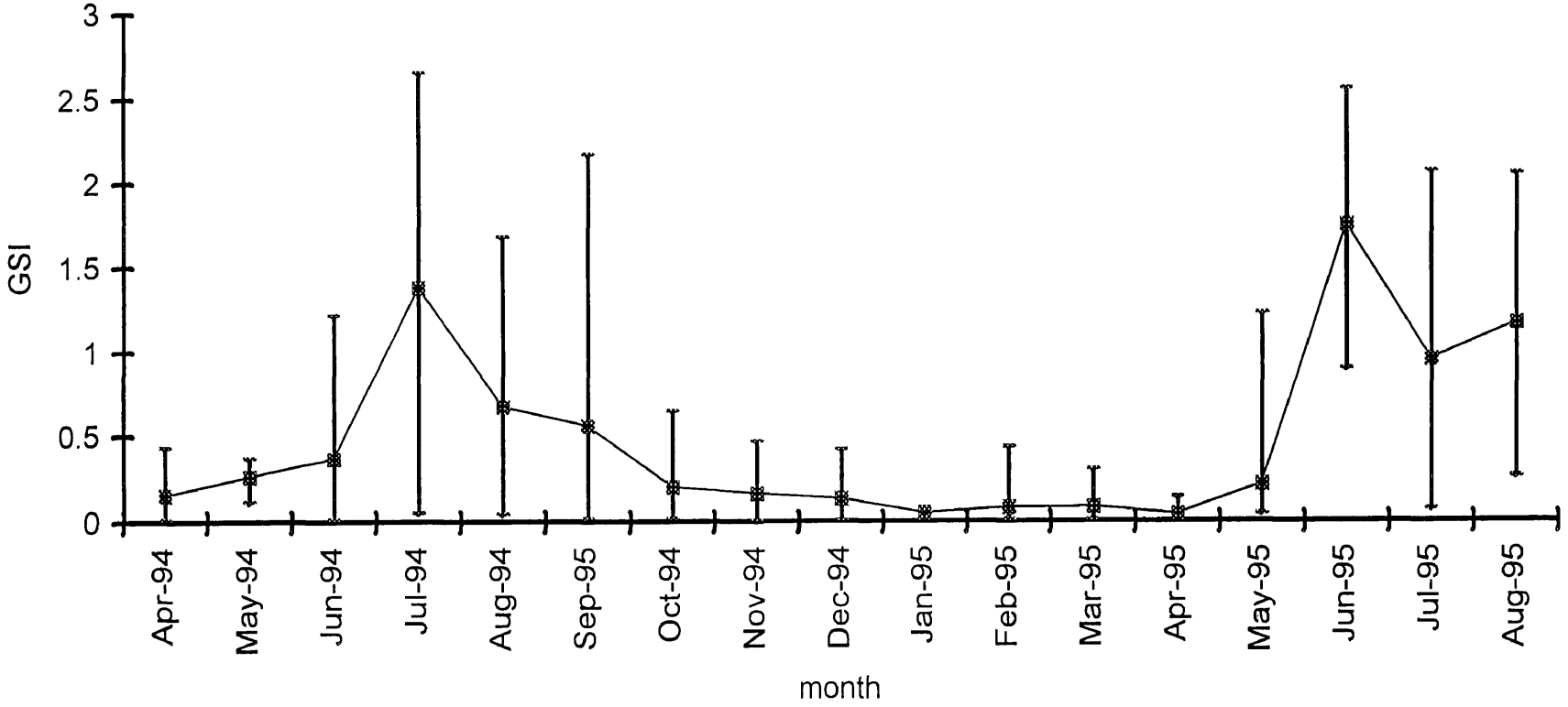

0.12
0.1
0.08
0.06
0.04
0.02
0
GSI
Apr-94
Jun-94
Aug-94
Oct-94
Dec-94
Feb-95
Apr-95
Jun-95
month

Figure 27. Monthly mean gonadosomatic indices for female blackcheek tonguefish, *Symphurus plagiusa*, from Chesapeake Bay.

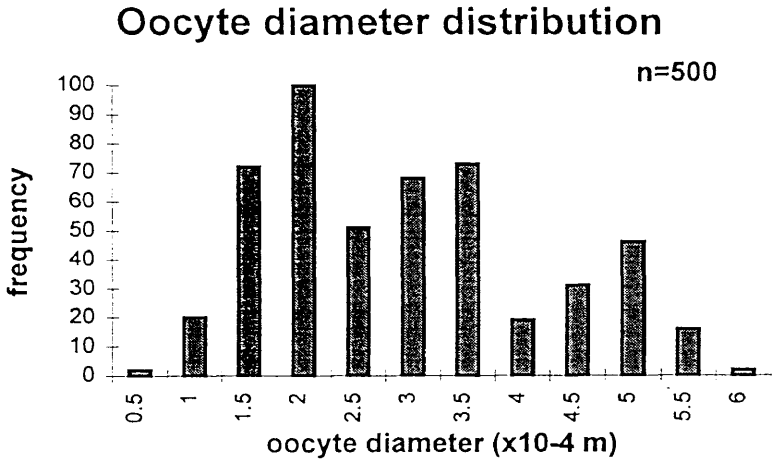

GSI
3
2.5
2
1.5
1
0.5
0
Apr-94
May-94
Jun-94
Jul-94
Aug-94
Sep-95
Oct-94
Nov-94
Dec-94
Jan-95
Feb-95
Mar-95
Apr-95
May-95
Jun-95
Jul-95
Aug-95
month

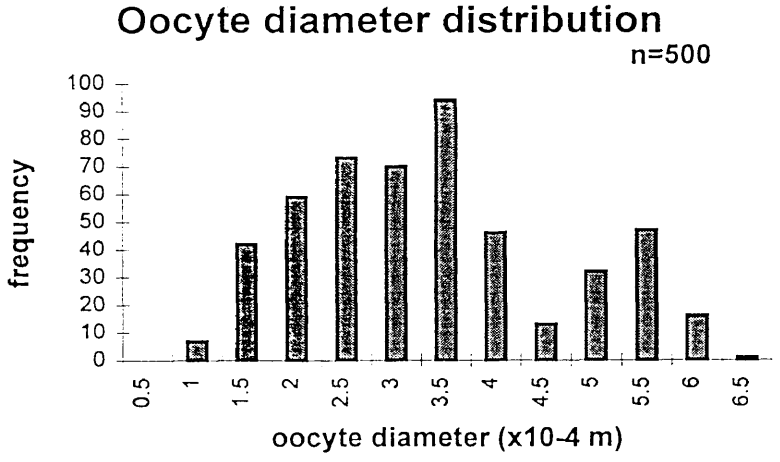
males in the June 1995 collection precludes the ability to narrow the GSI peak to one month during that year. High index values during these months correspond with the greatest occurrence in samples of mature, ripe individuals. GSI values for females were over an order of magnitude greater than those observed in males.

Although a 24-hr period could not be sampled, data indicate that blackcheek tonguefish spawn during daylight and early evening hours and more than once per day. Histological examination of female blackcheek tonguefish collected over a 12-hr period from 1020 to 2220 showed that spawning was imminent between 1020 and 1217 and again between 1629 and 1800, because ovarian sections contained hydrated oocytes. No hydrated oocytes were found in fish collected between 1300 and 1520 or between 1800 and 2220.

Blackcheek tonguefish are indeterminate, multiple spawners, which mature and release more than one batch of eggs over a protracted spawning season. Randomly selected oocytes taken from gravid females show continuous, bimodal size distributions, which are characteristic of indeterminate spawners (Fig. 28). Oocytes in the largest mode (>0.4 mm) represent those undergoing final maturation and hydration, while those in the smaller mode (<.04 mm) represent oocytes in various stages of early development. Oocyte diameters ranged from 0.05 mm to 0.65 mm. No gaps exist in either of the oocyte size distributions, which would be characteristic of species with determinate fecundity. The presence of partially spent individuals in the lower Chesapeake Bay during the spawning season indicates rapid turnover of oocytes in the ovary, and rapid maturation of less developed oocytes.

Figure 28. Oocyte diameter distribution of two gravid female blackcheek tonguefish, *Symphurus plagiusa*, from Chesapeake Bay.


Oocyte diameter distribution
n=500
frequency
100
90
80
70
60
50
40
30
20
10
0
0.5
1
1.5
2
2.5
3
3.5
4
4.5
5
5.5
6
oocyte diameter (x10-4 m)


Oocyte diameter distribution
n=500
frequency
100
90
80
70
60
50
40
30
20
10
0
0.5
1
1.5
2
2.5
3
3.5
4
4.5
5
5.5
6
6.5
oocyte diameter (x10-4 m)

Batch fecundity of female blackcheek tonguefish (116 mm-176 mm) ranged from 2846 to 16881 ova (Table 11). Mean batch fecundity was 7473 eggs per batch, standard error was 1239, and the 95% confidence interval was ± 2727. Batch fecundity was significantly related to both total length and total weight. The relationship between batch fecundity and total length appears to be exponential (Fig. 29), while the relationship between batch fecundity and total weight appears linear (Fig. 30). Batch fecundity is directly related to total weight. Data in the regression analysis of batch fecundity on total length were $\log_{10}$ transformed so that linear regression could be used to describe the relationship (Fig. 31). Batch fecundity increased significantly with both total length (ANOVA; n = 12, F = 16.90, P< 0.0025) and total weight (ANOVA; n=12, F = 68, P< 0.0005). The regression of number of ova on total length and total weight yielded the following equations:

$$\text{batch fecundity} = 10^{-4.22}\,(TL^{3.71})$$
$$\text{batch fecundity} = 287.08\ (\text{T.W.}) - 2224.6 \qquad (r^2 = 0.89)$$

Blackcheek tonguefish relative batch fecundity showed no statistically significant relation to total length (ANOVA; n = 12, F = .04, P>0.25), but there was a significant increase with total weight (ANOVA; n = 12, F = 5, P<0.05). Blackcheek tonguefish produce an average of 207.62 eggs/g female (± 11.98 SE) with estimated ranges from 170.84 eggs/g to 307.71 eggs/g.

Spawning frequency of individuals was estimated from the incidence of females with hydrated oocytes or oocytes undergoing FOM caught during the spawning period. The ovaries from 32 of 110 mature females (29%) caught during the spawning period (June

TABLE 11

Fecundity estimates of blackcheek tonguefish, *Symphurus plagiusa*, from Chesapeake Bay.

Collection date	TL (mm)	TW (G)	Batch Fecundity	Relative Fecundity	Spawns	Total fecundity
7/5/94	145	25.48	6474	254.08	38	246012
9/7/94	153	38.53	6829	177.24	38	259502
6/16/95	134	25.29	4745	187.62	38	180310
6/16/95	162	38.71	7967	205.81	38	302746
8/1/95	120	19.14	3270	170.85	38	124260
8/1/95	116	16.61	2846	171.34	38	108148
7/24/96	126	19.58	3881	198.21	38	147478
7/24/96	158	40.64	7020	172.74	38	266760
8/5/96	175	54.86	16881	307.71	38	641478
8/5/96	137	27.23	5104	187.44	38	193952
8/5/96	176	55.95	12385	221.36	38	470630
8/5/96	167	51.78	12275	237.06	38	466450
mean			7473	207.62		283977
standard error			1239	11.98		47056
95% CI			+/-2727	+/-26.37		+/-103570

Figure 29. Regression of batch fecundity on total length of female blackcheek tonguefish, *Symphurus plagiusa*, from Chesapeake Bay.

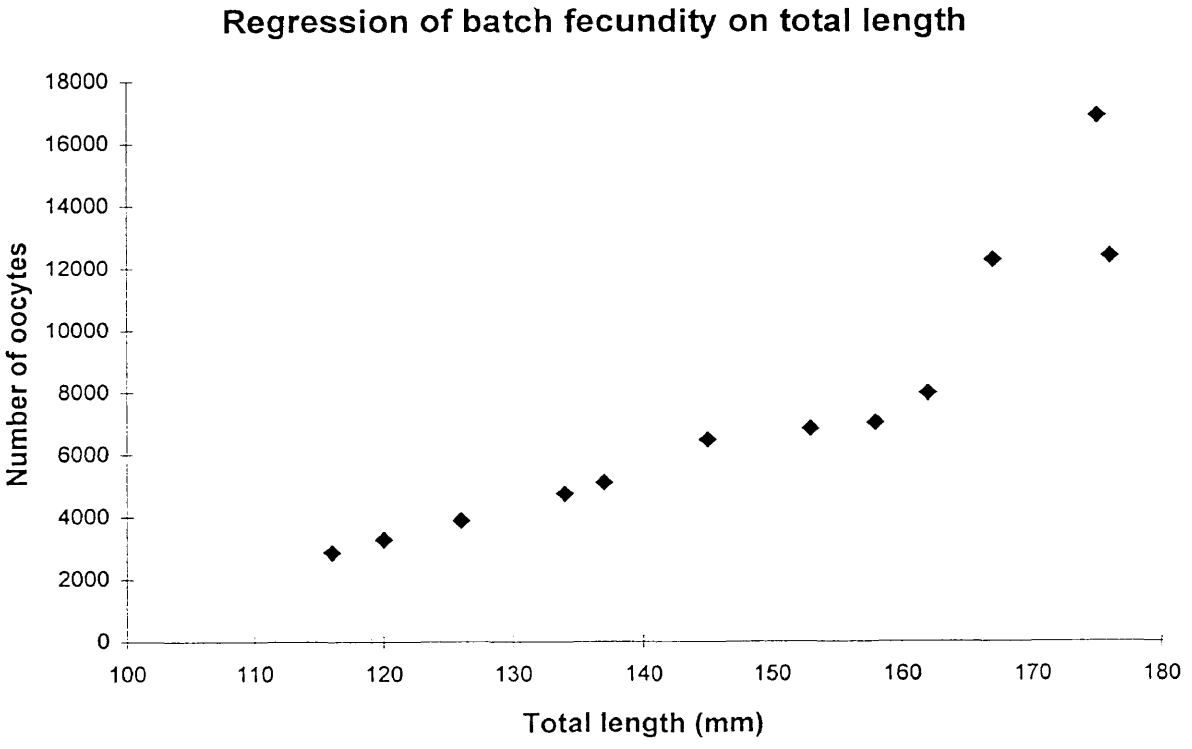

Regression of batch fecundity on total length
Number of oocytes
18000
16000
14000
12000
10000
8000
6000
4000
2000
0
100
110
120
130
140
150
160
170
180
Total length (mm)

Figure 30. Regression of batch fecundity on total weight of female blackcheek tonguefish, *Symphurus plagiusa*, from Chesapeake Bay.

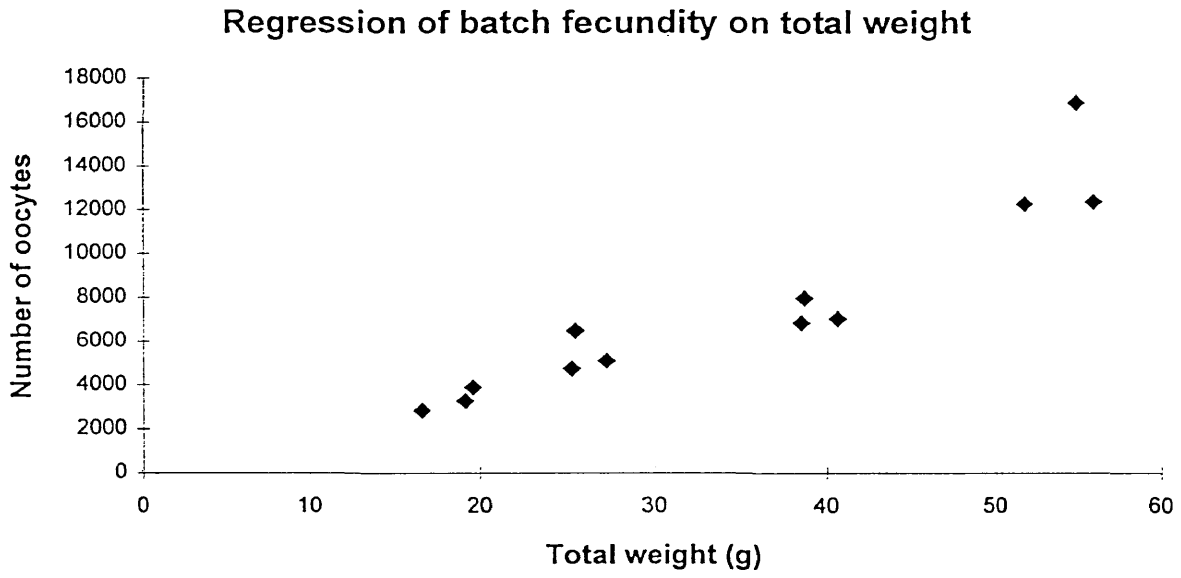
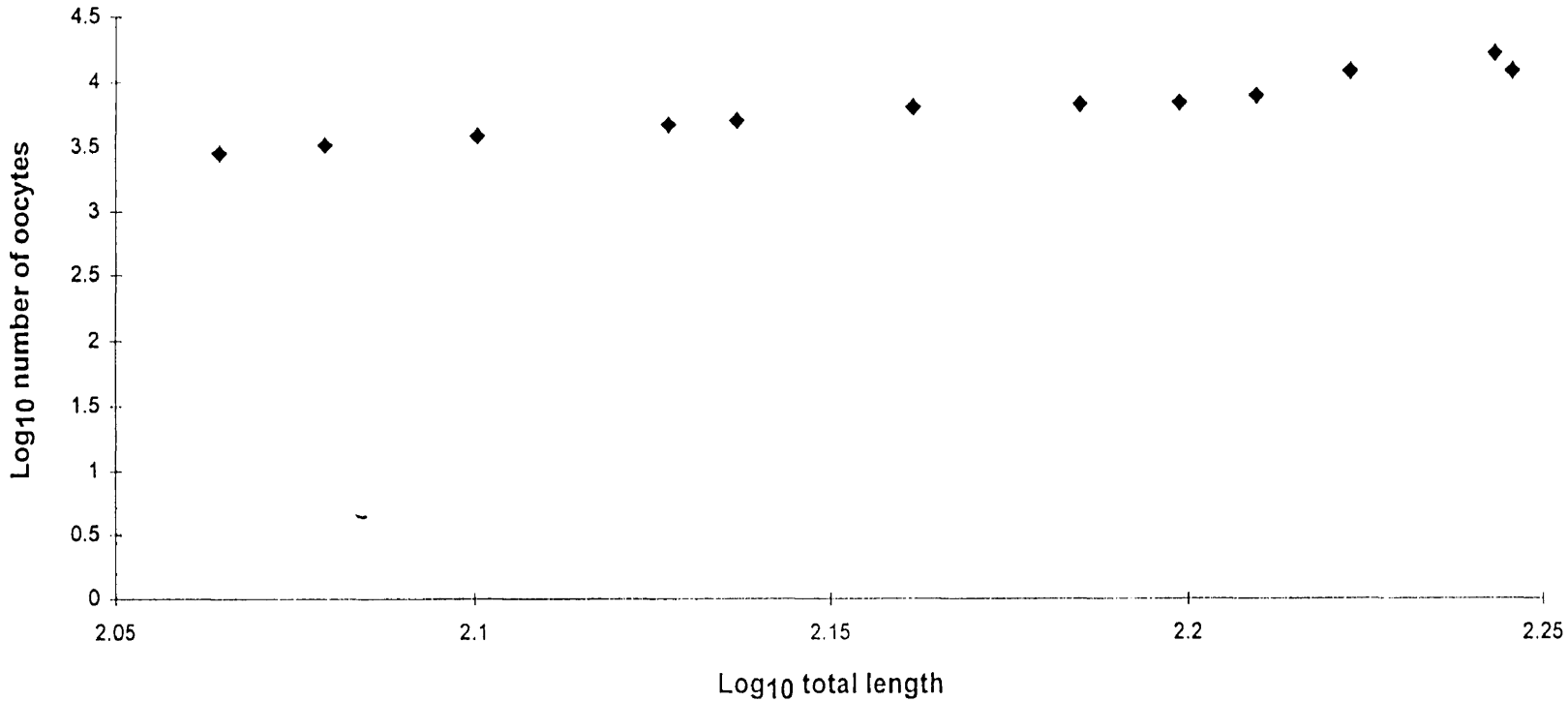

Regression of batch fecundity on total weight
Number of oocytes
18000
16000
14000
12000
10000
8000
6000
4000
2000
0
0
10
20
30
40
50
60
Total weight (g)

Figure 31. Regression of batch fecundity on total length of female blackcheek tonguefish, *Symphurus plagiusa*, from Chesapeake Bay: $\log_{10}$ transformed data.

Regression of batch fecundity on total length



through early October) had oocytes in these maturity stages. Thus, on average, female blackcheek tonguefish in Chesapeake Bay are estimated to spawn once every 3.4 days. For a spawning period of approximately 129 days, that is from 1 June through 7 October, a female blackcheek tonguefish could spawn an average 38 times.

Total annual fecundity, the product of batch fecundity and spawning frequency, ranged from 108148 eggs for a 116 mm female to 641478 eggs for a 175 mm female. Mean total fecundity was 283977 eggs, standard error was 47056, and the 95% confidence interval was $\pm$103570.

DISCUSSION

This is the first detailed study presenting information on reproductive biology of blackcheek tonguefish. Previous studies describing gonadal development during maturation consisted only of macroscopic gonadal examination (Hildebrand and Cable 1930; Moe and Martin 1965; Topp and Hoff 1972). Determination of maturity stages based upon macroscopic examination involves a degree of subjectivity on the part of the examiner. Histological examination of gonadal tissue, along with macroscopic examination and staging, allows for the most exact descriptions of gonadal maturity.

Histological examination of gonadal tissue shows that females have a typical teleostean gonad, while males have an atypical gonadal structure. Most fishes fall into one of two categories of gonad structure. In the restricted spermatogonial testis, spermatogonia are restricted to the distal terminus of each seminiferous tubule. Most teleosts have an unrestricted spermatogonial testis, in which the spermatogonia are distributed along the entire length of the seminiferous tubule (Grier 1981). Blackcheek tonguefish appear to have testes of the restricted type, with the following modifications. Typically, in fishes with a restricted type of testis, spermatogenesis occurs from the periphery of the testes inward, with Sertoli cells forming the borders of cysts in which synchronous spermatogenesis occurs. Blackcheek tonguefish differ in this pattern in that testes show

no such progression of spermatogenesis. Spermatogonia, spermatocytes, spermatids, and spermatozoa exist mixed together in the seminiferous tubules. The blackcheek tonguefish testes also contain large numbers of Leydig cells (Grier, pers. comm.), whose primary function is testosterone secretion. The presence of cell types other than spermatozoa in the peripheral sperm ducts cannot be explained. Given the extreme reproductive diversity found among the teleosts, it is not surprising that these variants were found (Grier, pers. comm.). Histological analyses of other species are necessary to determine if this pattern is typical for cynoglossids.

While it was possible to macroscopically classify six ovarian maturation stages for blackcheek tonguefish, histological processing of ovarian tissue revealed seven distinct maturity stages. Histological examination allowed for the macroscopic description of a developing ovary to be divided into an early and late stage. These subtle differences at the cellular level are not manifested as distinct changes at the whole gonad level.

The ovarian cycle of blackcheek tonguefish is similar to other multiple-spawning fishes. The ovarian cycle can start with an immature female, which enters the cycle for the first time by reaching sexual maturity, or with a resting adult female, which enters the cycle by entering the developing stage of oocyte maturation at the beginning of each spawning season. Females, after undergoing oocyte maturation to become fully developed, go through a secondary cycle which is observed in other multiple-spawning fishes, and characterizes blackcheek tonguefish as multiple, indeterminate spawners. During this secondary cycle, fully developed females cycle through the gravid stage by undergoing final oocyte maturation, hydration and spawning. If spawning has not been

completed, remaining advanced yolked oocytes are resorbed and a new batch of advanced yolked oocytes is recruited from the group of partially yolked oocytes. This cycle repeats until spawning is complete. After spawning is complete, females enter a regressing stage, where remaining oocytes become atretic and are resorbed, after which the ovaries return to the resting stage.

Sex ratio determined from samples collected in this study deviated from 1:1 due to large single-sex catches of females in the lower Bay during the summer months. Dense concentrations of female blackcheek tonguefish in spawning condition were collected in deeper, more saline areas of lower Chesapeake Bay during the spawning season. Hogchoker (*Trinectes maculatus*), a sympatric pleuronectiform species, was reported by Mansuetti and Pauly (1956) as displaying similar spatial separation of the sexes in the summer months prior to spawning, with females occupying grass bed and shoal regions. Dovel et al. (1969) found that hogchokers migrate within the Patuxent River estuary and spawn in more saline waters.

Length at 50% maturity (L_{50}) was determined by microscopic inspection of gonadal tissue. Males were considered mature if the testes contained spermatozoa, and females were considered mature if they were in a stage of development other than immature. Blackcheek tonguefish mature between the sizes of 80-130 mm TL for females and 70-110 mm TL for males. L_{50} calculated in this study (101 mm and 91 mm TL for females and males, respectively) corresponds to age 1 for both sexes. The range of sizes over which this species matures, coupled with the fact that most fish mature around age one, indicates that maturity relates better to age than to size. Roff (1982) hypothesized that in

early maturing species, genetic and/or physiological constraints (such as oocyte development time) may prevent a species from maturing at an earlier age. The size and age at first maturity seems to be a compromise that meets the needs of reproduction, growth, and survival (Stearns 1976; Roff 1991; Armstrong and Starr 1994). The small size and young age at sexual maturity for blackcheek tonguefish and hogchoker are factors that may account for the high abundances of these species in temperate estuaries where salinity, temperature, predation, and food availability are highly variable and may be stressful or limiting.

Blackcheek tonguefish caught in this study spawn in Chesapeake Bay from June through early October, coinciding with periods of seasonal thermal maxima. GSI for both sexes indicate peak spawning occurred in late June and early July. Although GSI may not yield a completely accurate indication of gonadal activity (DeVlaming et al. 1982), these findings agree with previous studies which estimated timing of spawning season from occurrence of eggs and larvae in plankton collections. Olney and Grant (1976) collected *S. plagiusa* larvae in Chesapeake Bay from July through September, with peak abundance in late July and August. Hildebrand and Cable (1930) reported *S. plagiusa* larvae in North Carolina waters from late May through early October, with dense concentrations caught in June. Although Hildebrand and Cable (1930) claimed that nearly all spawning occurred at sea, collections of *S. plagiusa* collected off western Florida led Topp and Hoff (1972) to believe that recruitment for this species onto the continental shelf occurred from migration of fish from inshore areas of Tampa Bay. The occurrence of early and late larval stages of blackcheek tonguefish in Chesapeake Bay led Olney and Grant (1976) to emphasize that

large estuaries may be significant spawning areas for the species. Mature females with hydrated oocytes were found only around the mouth of Chesapeake Bay in relatively deep, saline water, indicating that spawning occurs at the mouth of Chesapeake Bay. Individuals in spent and resting condition were also found in lower Chesapeake Bay, indicating that some individuals remain in the Bay proper after spawning; however, collections of larger individuals over the continental shelf (Wenner and Sedberry 1989; Munroe 1987) indicate that blackcheek tonguefish may emigrate from the Bay system onto the adjacent shelf.

Blackcheek tonguefish are indeterminate spawners that release many batches of eggs over a protracted spawning period. Oocytes in all maturity stages are found in developed ovaries, and no distinct hiatus in oocyte size frequency exists. Under certain conditions, especially those in highly unpredictable dynamic environments, multiple spawning fishes are hypothesized to have several advantages over total, or isochronal, spawners (McEvoy and McEvoy 1992). Serially spawning fishes have increased fecundity. When oocytes hydrate, they increase dramatically in size. If all oocytes in the ovary hydrate at the same time, restricted space could limit fecundity; however, if small batches of oocytes are hydrated over a protracted period, greater overall fecundity may result. Serial spawning also reduces the risk of egg and larval mortality by spreading the risk of predation on eggs and larvae over a longer period, by spreading the risk of spawning eggs over unfavorable climatic or feeding conditions, and potentially reduces inter- and intra-specific competition for prey items. Cushing (1975) hypothesized that serial spawning over a protracted period ensures that some larval cohorts hatch during those periods when conditions favor high

rates of survival. Serial spawning is common in fishes inhabiting tropical and warm temperate latitudes, where predation tends to be higher and food availability lower than in the higher latitudes. Serial spawning therefore appears to be the best mode of reproduction for fishes that occupy seasonally-dynamic temperate estuarine systems. Smith et al. (1975) and Miller et al. (1991) found that the bothids, soles, and tonguefishes of the tropical and temperate regions display extended spawning periods coupled with batch production of eggs and serial spawning, while the Pleuronectidae from high latitudes are generally characterized as being determinate spawners with relatively short spawning periods.

Spawning stock estimates are difficult in multiple spawning fishes because they require information on the number of batches of eggs released and the fecundity of each batch (McEvoy and McEvoy 1992). In blackcheek tonguefish, fecundity cannot be reliably estimated from counts of mature oocytes in the standing crop, nor can the number of batches produced each year be determined by the number of size classes in an oocyte size-frequency. However, batch fecundity is generally easy to calculate for blackcheek tonguefish. Ovaries are of a size small enough so that all hydrated oocytes in an ovary can be counted in under two hours. Hydrated oocytes are easily distinguishable from maturing oocytes due to their large size and clear appearance. By counting all hydrated oocytes in a sample, one can avoid introducing biases associated with statistical analyses used to determine positional differences in egg distribution within an ovary. Counts of all hydrated oocytes are also a better estimate of batch fecundity than counting a smaller percentage of hydrated oocytes and projecting that number to reflect total weight of the

gonad.

Spawning frequency is best estimated from the incidence of day-old post-ovulatory follicles (Hunter and Macewicz 1980). The lack of post ovulatory follicles in histological ovary sections of female blackcheek tonguefish indicates that this cellular stage is extremely ephemeral , and that these structures are resorbed rapidly after spawning. The estimate of spawning frequency, therefore, was determined by employing a hydrated oocyte method, also described by Hunter and Macewicz (1985). This method allows for spawning frequency estimates to be made using macroscopic examinations of gonadal tissue (after verification with histological methods). While this method is simpler than aging post-ovulatory follicles, it has some disadvantages. Sampling females with hydrated ovaries can only be done during a limited portion of each day; incidence of females with hydrated oocytes is contagiously distributed among fish samples; and females with hydrated oocytes may be more vulnerable to trawls. However, the simplicity of the method, as well as the absence of post-ovulatory follicles in ovaries, makes this method of estimating spawning frequency appealing.

There is evidence that several fish species exhibit declines in egg size during the course of the spawning season, perhaps due to increasing water temperatures and shorter incubation times later in the season (Ware 1975), or due to reduced food intake during the spawning season (McEvoy 1989; Kjesbu et al. 1990). Reduced egg sizes in batches spawned later in the season may affect total fecundity estimates. No progressional reduction in egg size with spawning season was noted for blackcheek tonguefish, although it has been observed for sole, *Solea solea* (Houghton et al. 1985) and turbot,

Scophthalmus maximus (Bromley et al. 1986).

Comparisons of reproductive parameters among cynoglossids are limited because few reproductive studies on this family are available (Table 12). Only two studies detailing reproductive parameters exists for symphurine tonguefishes, *S. atricauda* and *S. ligulatus*. *The Symphurus atricauda* study indicated that this species spawned three times over a spawning season of undetermined length (Goldberg 1980), while the *S. ligulatus* study (Cau and Deiana 1979) indicated determinate spawning and small size/late age at maturity. Studies on four species of *Cynoglossus*, (*C. macrolepidotus, C. dubius, C. arel, and C. lida*) show that members of this genus have similar reproductive traits that are different from those of symphurine tonguefishes. All four species spawn once per year during a short period. These species all mature at a larger size than that observed in symphurine tonguefishes. *Cynoglossus macrolepidotus* matures at 191 mm (Ramanathan et al. 1980), *C. dubius* at 246 mm (Seshappa 1974), *C. arel* at 172 mm, and *C. lida* at 158 mm (Rajaguru 1992). Small total size and small size at maturity are characteristic of symphurine tonguefishes (Munroe 1987).

North temperate estuaries such as Chesapeake Bay are rigorous physical systems known for environmental extremes in temperature, salinity, and dissolved oxygen, and with strong seasonal cycles of primary and secondary production. Representatives of five different pleuronectiform families (Cynoglossidae, Achiridae, Paralichthyidae, Scophthalmidae, and Pleuronectidae) inhabit Chesapeake Bay. This assemblage includes representatives of cold temperate and warm temperate flatfishes. Chesapeake Bay occurs at the northern end of a warm temperate region and at the southern end of a cold

TABLE 12

Comparison of reproductive parameters among symphurine and cynoglossid tonguefishes.

	Mode	Season	size/age @ maturity	Source
S. plagiusa	indeterminate	Jun-Oct	♀ = 95 mm/age 1 ♂ = 93 mm/age 1	Terwilliger 1996
S. atricauda	indeterminate	?????????	?????????????	Goldberg 1980
S. ligulatus	determinate	June-Nov	♀ = 58 mm/age 3 ♂ = 42 mm/age 2	Cau & Deiana1979
C. macrolepidotus	determinate	Aug-Oct	♀ = 191 mm/???	Ramanathan et al. 1980
C. dubius	determinate	Nov-Jan	♀ = 287 mm/???	Seshappa 1974
C. arel	determinate	Jun-Mar	♀ = 225 mm/age2 ♂ = 217 mm/age2	Rajaguru 1992
C. lida	determinate	Feb-Nov	♀ = 179 mm/age2 ♂ = 167 mm/age2	Rajaguru 1992

temperate region. The unique geographical location of the Bay may account for the presence of both warm and cold water groups occurring there. The diverse families of pleuronectiforms inhabiting Chesapeake Bay makes for an interesting comparison of reproductive aspects of the life history of these fishes. Six species found in Chesapeake Bay are blackcheek tonguefish and hogchoker, which complete their life histories in Chesapeake Bay, and summer flounder (*Paralichthys dentatus*), windowpane (*Scophthalmus aquosus*), winter flounder *(Pseudopleuronectes americanus*) and southern flounder (*Paralichthys lethostigma*), which do not complete their life cycle within Chesapeake Bay.

Although there are several published reproductive studies for non-commercial Pleuronectiformes occurring in temperate estuaries of the western north Atlantic, most are descriptive in nature, and few report histological gonadal staging or total fecundity estimates. Therefore, comparisons of fecundity parameters of blackcheek tonguefish with sympatric pleuronectiforms are limited because previous studies of indeterminate spawners provide only estimates of batch fecundity. Because fecundity is related to fish length and weight, relative fecundity measurements are necessary to make comparisons between species. Unfortunately, only estimates of these parameters for summer and winter flounder are available. Summer flounder relative batch fecundity estimates range from 1,077 to 1,265 eggs/g. These fish spawn approximately six batches of pelagic eggs over the course of the spawning period (Morse 1981). In comparison, blackcheek tonguefish relative batch fecundity ranges from 171 to 308 eggs/g. These fish spawn approximately 38 batches of pelagic eggs over the course of the spawning period. Although summer

flounder have higher relative fecundity than does blackcheek tonguefish, total annual fecundity is less because blackcheek tonguefish spawn many more times during this protracted spawning period. The winter flounder represents another reproductive pattern. This species is a total spawner which spawns demersal eggs once a year (Saila 1963). Winter flounder total fecundity ranged from 442 to 1,273 eggs/g. Fecundity may be relatively low in total demersal spawners because demersal eggs are hypothesized to be better protected against predation and dispersal away from nursery areas is less than for pelagic eggs.

Sympatric pleuronectiforms inhabiting Chesapeake Bay also exhibit considerable variety in age and size at maturity. Blackcheek tonguefish mature at the youngest age (1 year) and the second smallest size (96 mm) when compared to sympatric pleuronectiforms. Hogchokers mature at a smaller size (70 mm) and a slightly older age (2 years). In comparison, summer flounder mature at 2 years and 320 mm, southern flounder at 2-3 years and 340 mm, windowpane at 3-4 years and 230-250 mm, and winter flounder at 2-9 years and 250 mm.

The representatives of these five different families of flatfishes display wide variety in their reproductive parameters. Reproductive parameters in fishes are regulated by genetic and environmental factors. The relationship among habitat, life-history strategies, and population parameters has been termed *r* and *K* selection. The comparative nature of *r* and *K* selection is due to the fact that the continuum is an idealized model, and no species can be *r* or *K* selected in an absolute sense. Under an environmental regime with a large component of unpredictable, non-selective mortality, an organism should allocate more

energy towards ensuring reproductive success. Early age and small size at maturity, high fecundity, and indeterminate spawning indicate *r*-selected features. *K*-selected features include later age at maturity, larger size at maturity, lower fecundity, and determinate spawning. These features, exhibited by organisms which experience predictive, selective mortality, increase individual fitness at the expense of reproductive activity (Adams 1980). Blackcheek tonguefish reproduce at a younger age and smaller size, and have a higher total fecundity than other pleuronectiforms in this dynamic estuarine system. The other species are relatively K-selected in their reproductive parameters because they mature at a larger size and later age and exhibit relatively lower total fecundity than does *S. plagiusa*. Interesting to note is that only the small sized and short lived species (*S. plagiusa and T. maculatus*) complete their life cycles in Chesapeake Bay. Species with relatively r-selected life history parameters are hypothesized to be better adapted at exploiting seasonally dynamic and highly unpredictable environments. Many of the reproductive features of small-sized flatfishes completing their life histories within Chesapeake Bay are relatively r-selected, which is consistent with this theory.

SUMMARY OF CONCLUSIONS

- This is the first in-depth study on age, growth, and reproductive biology for blackcheek tonguefish, *Symphurus plagiusa*, as well as for any member of the genus *Symphurus*.
- Blackcheek tonguefish are represented in Chesapeake Bay by six year classes. Overlap in size at age indicates that obtaining year-class data from length frequencies alone is misleading concerning the information about age structure of the population.
- Blackcheek tonguefish grow rapidly their first year, and slower after age 1, when maturation occurs. Growth coefficients are high; therefore, blackcheek tonguefish reach asymptotic length quickly (about 190 mm TL, at age 5).
- There are seven stages of ovarian development. Blackcheek tonguefish are multiple spawners with asynchronous oocyte development and indeterminate fecundity. Estimated batch fecundity ranged from 2846-16881 ova in females ranging in size from 116-175 mm TL. Females are capable of spawning an average of 38 times over the spawning period; therefore, total annual fecundity for specimens ranged from 108,148 to 641,478.

- There are two maturity classifications for males. The atypical testes structure made staging males impossible; further analyses of gonad structure are necessary for complete understanding of male gonad structure and development.

- Blackcheek tonguefish spawn proximal to the mouth of Chesapeake Bay from June through October in deep, saline waters. This spawning may come at the end of an ontogenetic movement from shallow tidal creeks to deep, open embayments.

- When compared to sympatric pleuronectiforms, blackcheek tonguefish have a relatively high mortality rate, small asymptotic length, high growth rate, younger age at maturity, and higher total annual fecundity. Blackcheek tonguefish appear to have life history characteristics that lie on the r-end of the r and K continuum.

- Chesapeake Bay is the northernmost outpost of a significant population of blackcheek tonguefish that may exhibit entirely different life history parameters than other populations distributed through the species' range. Once determined, life history parameters of northern fish can be used to compare with other populations at southern parts of the range, as well as to compare with the life histories of other flatfish species.

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